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MELBOURNE

THE EFFECT OF LIGHT ON THE MATING BEHAVIOUR OF THE QUEENSLAND FRUIT FLY *STRUMETA TRYONI* (FROGG.)

By L. Barton Browne*

[*Manuscript received December* 5, 1956]

Summary

The effects of light during both the dusk and day periods on the mating behaviour of *Strumeta tryoni* (Frogg.) have been studied.

In nature the flies mate only at dusk. The effects of several components of dusk illumination were examined. The area of the source of artificial dusk and the distance between it and the flies are not important so long as the angle subtended by the source exceeds 25°, below which sexual activity is reduced. The illuminance in the cages and not the luminance per unit area (brightness) of the source determines the amount of sexual activity. The higher the illuminance during the day period the higher is the optimal illuminance at dusk. The rate of change from day to dusk appears to be unimportant.

Light during the day periods influenced the age at which both females and males became sexually active. For females only the effect of daily period was studied. At 240 lm/sq. ft those receiving light for 2 hr/day became sexually active later in life than those receiving light for 7½ hr/day. The age at which females become active is probably controlled by the state of the ovaries, whose rate of development is influenced by light. Males become active later in life when kept under 120 lm/sq. ft for 7½ hr/day than when kept under either 60 or 240 lm/sq. ft for the same daily period. Those receiving 240 lm/sq. ft for 2 hr/day become active later in life than those receiving this illuminance for either 4 or 7½ hr/day. Since light does not influence the development of the testes, it is probable that light directly affects the mating behaviour of the males. Light also influences the time at which the males begin and end sexual activity each day and the period for which they remain active. Those under 120 lm/sq. ft begin and end activity later than those under either 60 or 240 lm/sq. ft and remain active for a shorter period. The greater the daily period of illumination the earlier do they begin and end activity. At 240 lm/sq. ft those illuminated for 7½ hr/day remain active longer than those illuminated for 4 or 2 hr/day.

I. INTRODUCTION

Light has been shown to influence mating in the Queensland fruit fly *Strumeta tryoni* (Frogg.) in two ways. The flies mate only at dusk (Myers 1952) and the illuminance and the daily period of light to which the flies are exposed affects the age at which the flies mate and the frequency of mating (Barton Browne 1956). This paper reports some experiments which were done to investigate the ways in which light during both dusk and day affected mating.

Myers (1952) described the mating behaviour of the fruit fly in some detail. A brief description is included here. Flies become sexually active only under low illuminance and then only after they have been previously exposed to a high illuminance. Males respond to a suitable low illuminance by an increase in general

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activity and by the periodic emission of a high-pitched call. This call is produced by the rapid stridulation of the anal lobes of the wings against the abdominal combs (Monro 1953). The response of the females to dusk is an increase in the amount of flying and running. The call of the male stimulates the female to approach and after some manoeuvering for position, which does not appear to follow a set pattern, copulation is attempted.

In the experiments to be described, the criterion used for male sexual activity was the act of calling. The number of males active in a cage was determined by direct observation, the vibration of their wings being easily seen. No such obvious criterion can be obtained for sexual activity of females. The amount of activity shown by males was therefore used in most experiments as a measure of the relative abilities of various light treatments to stimulate the flies to become sexually active.

II. Methods

The methods used for breeding the flies, and for controlling temperature, humidity, and light were described in a previous paper (Barton Browne 1956) as were the "Perspex" cages used. Papaw (*Carica papaya*) mixture (Myers 1952), sugar, and water were provided unless otherwise stated. The flies were exposed to natural dusk by placing them in front of a window in the southern wall of the constant temperature room 10 min before the official time of sunset.

Artificial dusk illumination was produced by placing white paper screens in front of the fluorescent tubes used for lighting (see Barton Browne 1956). The screens occupied the entire cross section of a shelf. Differences in luminance of the outermost screen were obtained by altering the number of screens between it and the tubes and by altering the distances between the screens and between the innermost screen and the tubes. All natural light was excluded from the room.

The dusk illuminances referred to are comparative and are, unless otherwise stated, readings (units) given by a Weston Master I photo-exposure meter at a distance of 2 in. from the centre of the outermost screen. An omnidirectional photometer would have been necessary to obtain absolute values. Moreover, absolute values would have had little meaning relative to the day illuminances as the screens changed the quality of the light to a yellow-green.

An index of sexual activity of males under artificial dusk was obtained in the following way. Observations were started 1 min after the flies were exposed to artificial dusk illumination and were made at 2-min intervals until the period of exposure was 15 min. The number of active males in each cage was counted at each observation. The mean sum of the numbers of flies observed to be active per cage in these eight observations was called the "index of activity".

III. Results

(a) *Components of Dusk Illumination which May Affect Mating Behaviour*

(i) *The Size of the Source of Dusk Illumination and the Distance between It and the Cages.*—The indices of activity were determined when cages containing eight males and eight females were placed at distances of 2, 4, or 8 in. from sources of dusk illumination 14 or 1¾ in. high whose length was effectively infinite. The vertical angle subtended by the light source at the surface of the cages nearest it varied with both distance and height. The value of this angle for each combination of height and distance is given in Table 1. In one series of experiments the luminance per unit area (brightness) of the source was kept constant such that the photo-exposure meter read 0·6 lm/sq. ft when placed in contact with the centre of the outermost screen. In a second series the maximum illuminance within the cages was kept constant by adjusting the luminance per unit area of the outermost screen so that the photo-exposure meter gave a maximum reading of 0·6 lm/sq. ft when in the vertical plane of the surface of the cage nearest the source. The flies used were in their 6th week of adult life and had been exposed to 240 lm/sq. ft for 7½ hr/day.

TABLE 1

VERTICAL ANGLE SUBTENDED BY THE SOURCE AT THE SIDE OF THE CAGE NEAREST TO IT WHEN THE HEIGHT OF THE SOURCE AND THE DISTANCE FROM THE SOURCE TO THE CAGES VARIED

Height of Source (in.)	Distance between Source and Cage (in.)	Angle
14	2	148° 6′
14	4	120° 30′
14	14	53° 8′
1¾	2	47° 16′
1¾	4	24° 42′
1¾	14	7° 8′

TABLE 2

EFFECT OF THE HEIGHT OF THE SOURCE AND THE DISTANCE BETWEEN THE SOURCE AND THE CAGES ON THE INDEX OF ACTIVITY WHEN THE LUMINANCE PER UNIT AREA OF THE SOURCE REMAINED CONSTANT

The effects of height and distance are significant ($P < 0{\cdot}01$). Means whose logarithms of $(x+1)$ differ by more than 0·14 are significantly different at the 0·05 level

Height of Source (in.)	Distance between Source and Cages (in.)					
	2		4		14	
	Index of Activity	Coeff. of Variability (%)	Index of Activity	Coeff. of Variability (%)	Index of Activity	Coeff. of Variability (%)
14	24·3	21·3	19·0	19·4	15·3	26·1
1¾	14·5	28·4	9·2	29·2	0·0	—

Table 2 shows that when the luminance per unit area was kept constant the index of activity decreased (as did the maximum illuminance within the cages) with both increasing distance and decreasing height. Table 3 shows that when the illuminance was constant the index of activity was the same at all distances when the screen was 14 in. high, but decreased as the cages were moved away from screens only 1¾ in. high. The index of activity was not influenced by height when the distance from the source was only 2 in. but at greater distances decreased with decreasing height.

TABLE 3

EFFECT OF THE HEIGHT OF THE SOURCE AND THE DISTANCE BETWEEN THE SOURCE AND THE CAGES ON THE INDEX OF ACTIVITY WHEN THE ILLUMINANCE BETWEEN THE CAGES REMAINED CONSTANT

The effects of height and distance are significant ($P < 0{\cdot}01$). Means differing by more than 6·6 are significantly different at the 0·05 level

Height of Source (in.)	Distance between Source and Cages (in.)					
	2		4		14	
	Index of Activity	Coeff. of Variability (%)	Index of Activity	Coeff. of Variability (%)	Index of Activity	Coeff. of Variability (%)
14	23·2	21·9	24·0	19·8	24·8	23·1
1¾	24·8	19·9	17·6	24·6	0·0	—

Two conclusions were drawn from these results: (1) the index of activity depends upon the illuminance to which the flies are exposed and not upon the luminance per unit area of the source; and (2) neither the area of the source nor the distance of the source from the cages influences the index of activity provided that the vertical angle subtended by the source at the cages is sufficiently great and the illuminance in the cages is unaltered. At an angle of 25° there was a significant reduction, whereas at 7° no activity occurred.

(ii) *Rate of Change in Illuminance from High to Low.*—Five cages each containing eight males and eight females were exposed from the time of their emergence to 240 lm/sq. ft for 7½ hr/day. These flies fed on protein hydrolysate, sugar, and water. During the 5 weeks of the experiment a period of dusk was produced each day by covering the fluorescent tubes with four paper screens which extended the full length of the shelf but were only 3 in. in height and were 14 in. from the cages.* The photometer read 0·6 units when held 2 in. from the centre of the screen. On alternate days all four screens were placed in position at once. On the other days the screens were placed in position one at a time at 3-min intervals. After gradual reduction the flies were exposed to the final dusk illumination for 20 min. When the reduction was sudden, the flies were exposed to dusk for 25 min. The flies then were sexually active for approximately equal times.

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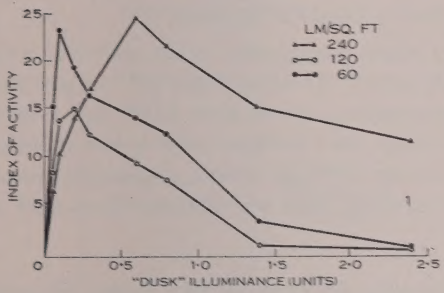
* This experiment was done before the vertical angle subtended by the source was recognized as a factor in dusk illumination. Flies fed on papaw mixture would not become active when the source was only 3 in. high and 14 in. from the cages, but other observations showed that flies fed on protein hydrolysate would become active. These were therefore used.

The number of copulations (observations made on all five cages each day), the number of attempted copulations (observations made on one cage selected at random each day), and the maximum number of sexually active males in each cage (observations made on one cage selected at random each day during weeks 3 and 4) were recorded.

The reduction in four stages was found to be no more effective in stimulating the flies to become sexually active than was the same reduction in one stage. It is probable, therefore, that even a continuous gradation in the reduction of illuminance, as occurs in the field, would not be more effective than a sudden reduction.



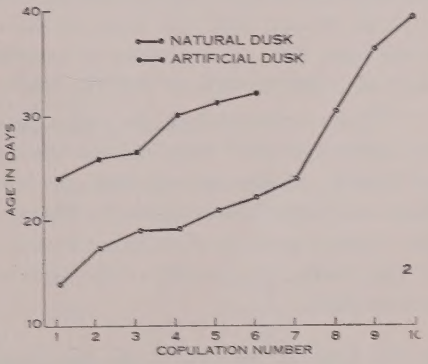


Fig. 1.—Indices of activity of male flies exposed to different combinations of day and "dusk" illuminances. The effect of day and dusk illumination and the interaction between the two are significant ($P<0{\cdot}01$). Means differing by more than 6·7 are significantly different at the 0·05 level.

Fig. 2.—Comparison of the effectiveness of artificial dusk with that of natural dusk. Age in days of flies when each copulation occurred in cages containing eight males and eight females. The effect of type of dusk is significant ($P<0{\cdot}01$). Means differing by more than 1·6 are significantly different at the 0·05 level.

(iii) *Illuminance.*—An experiment was done to determine whether the optimal dusk illuminance for the stimulation of male flies to become sexually active depended upon the illuminance to which the flies had been exposed during the day period.

Twenty cages, each containing eight males and eight females, were kept under an illuminance of 240 lm/sq. ft for 7½ hr, followed by dusk illumination (0·6 units) for ½ hr each day during the first 5 weeks of adult life. During the 6th, 7th, and 8th weeks* of life the flies were exposed to the various combinations of day (7½ hr) and dusk (½ hr) illuminances corresponding to the points in Figure 1. On every third day during these weeks the 20 cages were divided into four groups of five cages. Each group was exposed to one of the 30 combinations of day and dusk illuminances and the index of activity was determined. On the 2 days between observations, all the flies were exposed to 240 lm/sq. ft for 7½ hr and to 0·6 units for ½ hr so that all the flies might receive the same treatment during the 2 days before an observation was made.

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* The index of activity for flies kept under 240 lm/sq. ft during the day period and 0·6 units during the dusk period did not alter significantly during these weeks.

Figure 1 shows that the higher the illuminance during the day period the higher was the illuminance required at dusk to produce maximal values of the index of activity.

(iv) *Comparison of the Effectiveness of Artificial Dusk with that of Natural Dusk.*—Two groups of five cages each containing eight males and eight females were illuminated at 240 lm/sq. ft for 7½ hr/day during the first 7 weeks of adult life. One group of cages was exposed each day to natural dusk for 40 min beginning 10 min before sunset. The other group was exposed each day to artificial dusk of 0·6 units which was produced by a source 14 in. high at a distance 2 in. from the cages. The flies were left under artificial dusk for 40 min. The following were recorded: the ages at which 50 per cent. (i.e. four) of the male flies in each cage first became active at the same time; the numbers of successful copulations during the first 7 weeks of adult life; and the ages of the flies when each copulation occurred.

Under artificial dusk the mean age at which 50 per cent. of the male flies were first active was 25·2* days and the mean frequency of mating was 1·00** copulations per female. Under natural dusk the corresponding figures were 15·6* and 1·46**. These results and those given in Figure 2 show that artificial dusk is less effective than natural dusk in stimulating flies to become sexually active. A possible reason for this is that the quality of the light of artificial dusk was different from that of natural dusk.

(*b*) *The Effect of Light during the Day Period on the Age at which Flies Become Sexually Active*

Barton Browne (1956) showed that the age at which the flies mated was affected by light during the day period. The experiments described here were done to investigate the influence of light on the age at which the sexes first became sexually active.

(i) *The Age at which Males Became Active.*—Groups of five cages each containing eight males and eight females were kept from the time of emergence of the flies under the following illuminances and daily periods of illumination:

60 lm/sq. ft for 7½ hr /day
120 lm/sq. ft for 7½ hr/day
240 lm/sq. ft for 7½ hr/day
240 lm/sq. ft for 4 hr/day
240 lm/sq. ft for 2 hr/day
0 lm/sq. ft for 23½ hr/day

The flies were exposed to natural dusk each day. The first day on which four of the eight males in each cage became sexually active at the same time was recorded.

The results are given in Table 4. The age at which the males became active was influenced by illuminance and daily period. Males kept in constant darkness had not become active by the end of the 7th week of adult life. Those kept under 120 lm/sq. ft for 7½ hr/day became active much later in life than those under either 60 or 240 lm/sq. ft. At 240 lm/sq. ft they became active slightly earlier than at

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* Significantly different at the 0·05 level. ** Significantly different at the 0·01 level.

60 lm/sq. ft. The longer the daily period of illumination, the earlier did the male flies become sexually active.

(ii) *The Rate of Development of the Testes.*—Groups of five cages each containing 16 male flies were each exposed from the time of emergence of the flies to one of the following:

60 lm/sq. ft for $7\frac{1}{2}$ hr/day	240 lm/sq. ft for 4 hr/day
120 lm/sq. ft for $7\frac{1}{2}$ hr/day	240 lm/sq. ft for 2 hr/day
240 lm/sq. ft for $7\frac{1}{2}$ hr/day	Constant darkness

After 18 days the flies were dissected under "Drosophila Ringer's solution" (Ephrussi and Beadle 1936) and their testes were examined for the presence of active sperm. The results showed that the age at which the testes matured was not affected by light; the mean numbers of males per cage of 16 whose testes contained motile sperm when 18 days old only varied from 8·7 to 9·4. None of the differences between the means were significant at the 0·05 level. Evidently the differences in the amounts of food eaten (Barton Browne 1956) by males under these different illuminances and daily periods are not great enough to influence the rate of maturation of the testes. The age at which the males became active is not correlated with the state of the testes. Light must therefore affect the mating behaviour of the males in some other way.

TABLE 4

EFFECT OF LIGHT ON THE AGE AT WHICH MALE FLIES FIRST BECAME SEXUALLY ACTIVE

The effect of light is significant ($P<0{\cdot}01$)

Illuminance (lm/sq. ft)	Period of Illumination (hr/day)	Age at which 50% of Males were First Sexually Active	
		Mean	Coeff. of Variability (%)
60	7·5	17·6*	9·1
120	7·5	31·6	7·6
240	7·5	15·6*	8·3
240	4	17·8*	10·7
240	2	22·6	26·1

* Do not differ significantly at the 0·05 level.

(iii) *The Index of Activity of Males when Exposed to Optimal Dusk.*—An experiment was described above in which the indices of activity were determined when flies were exposed to different combinations of day and dusk illuminances (see Fig. 1). The indices of activity were different when flies were kept under different day illuminances and exposed to the dusk illuminances which was optimal for each. The maximum indices of activity for males under 60 and 240 lm/sq. ft, 23·2 and 24·4 respectively, were both greater ($P<0{\cdot}01$) than the index of activity of 15·0 of males under 120 lm/sq. ft. A similar result could not be obtained for the effect of the daily periods of illumination on the index of activity because, when males were exposed to illumination for a certain period on one day and for a different period on the next day, they would not become satisfactorily sexually active.

Light during the day period may have influenced the index of activity directly or by influencing the amount of feeding. Experiments were done to investigate these possibilities and to determine whether the illuminance throughout the day or that just prior to dusk determines the index of activity of males during the dusk period.

(iv) *Index of Activity of Males Deprived of Food during the Light Period.*—Flies were exposed from the time of their emergence to 240 lm/sq. ft for 7½ hr, followed by dusk of 0·6 units for ½ hr each day. For 1 day during the 6th week of adult life they were deprived of food and water at the beginning of the day period. On this day five cages each containing eight males and eight females were exposed to 120 lm/sq. ft for 7½ hr, followed by exposure for ½ hr to dusk of 0·2 units (optimal). A further five cages were exposed to an illuminance of 240 lm/sq. ft and to dusk of 0·6 units (optimal). The mean index of activity was determined for each series of cages.

The index of activity of the males under 240 lm/sq. ft, 21·3, was greater ($P<0{\cdot}05$) than that, 14·8, of those under 120 lm/sq. ft. The indices of activity did not differ significantly from those of flies which were not deprived of food. Light therefore directly affects the mating behaviour of the males; the amount of feeding during the day is not an important factor in determining the index of activity of the males.

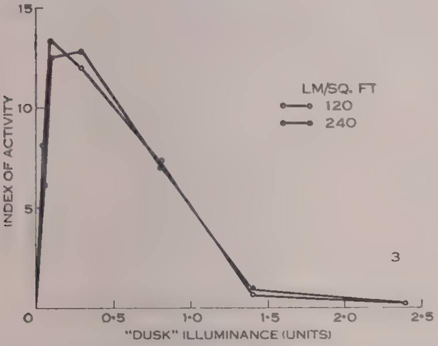
(v) *The Effect of the Illuminance just Prior to Dusk.*—Five cages each containing eight males and eight females were kept for the 5 weeks following the emergence of the flies under 240 lm/sq. ft for 7½ hr/day. On every 3rd day during the 6th, 7th, and 8th weeks the flies were exposed to an illuminance of 120 lm/sq. ft for 7¼ hr, then to an illuminance of 240 lm/sq. ft for ¼ hr, and finally to dusk for ½ hr. A different dusk illuminance was given each day that the flies were treated in this way. On the 2 days between observations, the flies were kept under 240 lm/sq. ft for the whole 7½ hr, followed by dusk of 0·6 units for ½ hr. This was done so that the flies received the same treatment during the 2 days before an observation was made.

Figure 3 shows that the indices of activity for males exposed to 120 lm/sq. ft for 7¼ hr/day and to 240 lm/sq. ft for ¼ hr/day did not differ from those of males exposed to 120 lm/sq. ft for the full 7½ hr. The index of activity and the optimal dusk depended upon the illuminance throughout the day and not on that during the ¼ hr before dusk. When flies were kept under an illuminance of 240 lm/sq. ft for 7¼ hr, followed by darkness for ¼ hr, they failed to become active when exposed to dusk. Some reduction in illuminance was therefore necessary.

The experiments in these sections showed that illuminance throughout the day in some way directly affects the sexual activity of the male flies.

(vi) *The Age at which Females Became Active.*—Female flies were kept from the time of emergence under 240 lm/sq. ft for either 7½ hr/day or for 2 hr/day. Five cages each containing eight females were exposed to each daily period of illumination.

Every day, 1 hr before the cages were exposed to natural dusk, eight sexually mature males which had been exposed to 240 lm/sq. ft for 7½ hr/day were introduced into each cage. The age of the females when each copulation occurred was recorded. Figure 4 shows that the matings occurred earlier in life when females were exposed to light for 7½ hr/day than when they were exposed for only 2 hr/day. The age at which females became sexually active was influenced by light during the day period.



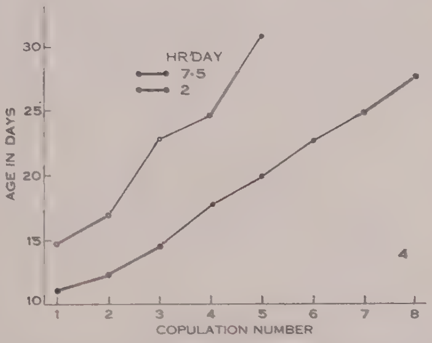


Fig. 3.—Effect of illuminance during the ¼ hr immediately before exposure to dusk. The indices of activity of flies under various dusk illuminances when they were exposed to (i) 120 lm/sq. ft for 7½ hr; (ii) 120 lm/sq. ft for 7¼ hr followed by 240 lm/sq. ft for ¼ hr. The effect of illuminance during the ¼ hr before dusk is not significant at the 0·05 level.

Fig. 4.—Effect of light on the age at which females become sexually active. The day of life on which each copulation occurred in cages of female flies kept under 240 lm/sq. ft for 7½ and 2 hr/day, when mature males were released into the cages each day 1 hr before exposure to dusk illumination. The effect of daily period of illumination is significant ($P < 0{\cdot}01$). Means differing by more than 5·0 are significantly different at the 0·05 level.

(vii) *The Rate of Development of the Ovaries.*—Light affects the rate of development of the ovaries; virgin and mated females develop eggs more rapidly when illuminated for 7½ hr/day than when illuminated for 2 hr/day at 240 lm/sq. ft (Barton Browne 1956). Either the age at which females become sexually active depends on the state of development of the ovaries or light affects some process which influences both sexual activity and development of the ovaries.

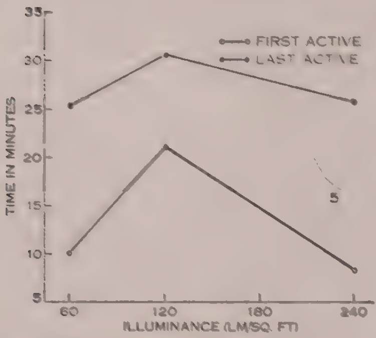
(c) *The Effect of Light during the Day Period on the Time at which the Flies Begin and End Sexual Activity during Exposure to Natural Dusk*

Experiments were done to determine whether the time at which male flies began and ended sexual activity, and the period for which they remained active under natural dusk, was influenced by light during the day period. Sets of five cages each containing eight males and eight females were kept from the time of emergence of the flies under the following illuminances and daily periods of illumination:

60 lm/sq. ft for 7½ hr/day
120 lm/sq. ft for 7½ hr/day
240 lm/sq. ft for 7½ hr/day
240 lm/sq. ft for 4 hr/day
240 lm/sq. ft for 2 hr/day

They were exposed to natural dusk each evening for 40 min beginning 10 min before the time of sunset. During the 6th week of life of the flies, the number of minutes which had elapsed after the initial exposure to dusk before four males were first and last seen active in each cage was recorded.

Figure 5 shows that flies exposed to 60 lm sq. ft or 240 lm sq. ft for 7½ hr day began and ended activity earlier than did those exposed to 120 lm sq. ft. Figure 6 shows that flies under 240 lm sq. ft for 7½ hr day began and ended sexual activity earlier each day than those exposed for 4 hr day; those exposed for 2 hr day were active even later than these. The time when the flies became active and the time when their activity ceased were strongly correlated.



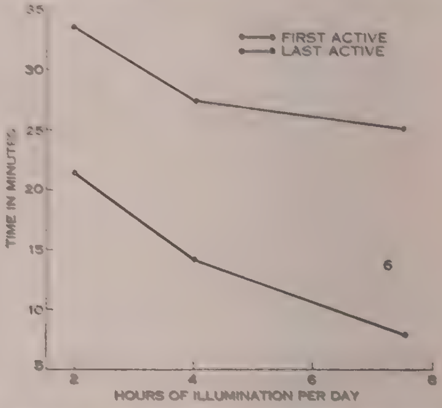


Fig. 5.—Effect of illuminance during the day period on the time when four males per cage were first and last sexually active and on the period for which they remained active during exposure to natural dusk. The effect of illuminance on both the period of activity and the time at which the flies were first active is significant at the 0·01 level; that on the time at which the flies were last active is significant at the 0·05 level.

Fig. 6.—Effect of daily period of illumination on the time when four males per cage were first and last sexually active, and on the period for which they remained active during exposure to natural dusk. The effect of illuminance on the time at which the flies were last active is significant at the 0·01 level; that on the period of activity and on the time at which the flies were last active is significant at the 0·05 level.

Illuminance and daily period of illumination also influenced the period for which the flies remained active. Flies under 120 lm sq. ft for 7½ hr day remained active for a shorter time each evening than those under 60 or 240 lm sq. ft for the same period (Fig. 5). Flies exposed to 240 lm sq. ft for 2 or 4 hr day remained active for a shorter time each evening than those exposed for 7½ hr (Fig. 6). Flies under 120 lm sq. ft for 7½ hr day and those under 240 lm sq. ft for 2 or 4 hr day had less chance of mating each evening than those under the other illuminances and daily periods.

The effect of illuminance during the day on the time at which activity began can be interpreted by referring to Figure 1 in which the indices of activity were given for male flies exposed to different combinations of day and dusk illuminances. This figure shows that at any dusk illuminances above 0·3 units, the indices of activity were highest for flies under 240 lm sq. ft, and next highest for those under 60 lm sq. ft and lowest for flies under 120 lm/sq. ft. It would therefore be expected that flies under 240 lm/sq. ft would become active first, that flies under 60 lm/sq. ft would become active second, and that those under 120 lm/sq. ft would become active last.

The following two experiments indicate some of the factors which cause the cessation of mating behaviour by males at the end of the dusk period.

(i) *The Effect of Very Low Illuminance on Mating Behaviour of the Males.*—Flies were exposed from the time of their emergence to 240 lm/sq. ft $7\frac{1}{2}$ hr day, followed by dusk illuminance of $\frac{1}{2}$ hr day. Ten cages each containing eight males and eight females were used. For 1 day during the 6th week of adult life the cages were divided into two groups. One group of five cages were retained under 240 lm/sq. ft; the other group of five were exposed to 120 lm/sq. ft. After they had been under these illuminances for $7\frac{1}{2}$ hr, the flies were exposed to the artificial dusk which was optimal for each. During the dusk period, the numbers of active males were counted in each cage every 2 min beginning after the flies had been exposed to dusk for 1 min. When in any cage the sum of the numbers of males observed to be active at each observation reached 20, the cage was transferred to a very low illuminance. (This was so low that the photo-exposure meter did not register.) The time taken after this transfer for activity of the males to cease was recorded for each cage. Male flies which had been under 240 lm/sq. ft ceased sexual activity 12·9 min after transfer to a lower illuminance whereas those which had been under 120 lm/sq. ft ceased after 20·4 min.

A low illuminance was more effective in causing activity to cease when male flies had been under 240 lm/sq. ft than when they had been under 120 lm/sq. ft. This was probably one of the reasons why the flies kept under 120 lm/sq. ft become inactive later in the natural dusk period than do those under 240 lm/sq. ft.

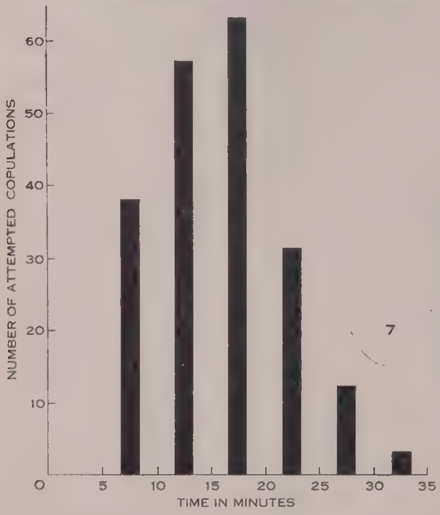
(ii) *The Period for which Males would Remain Active.*—Five cages each containing eight males and eight females were exposed from the time of emergence of the flies to 240 lm/sq. ft for $7\frac{1}{2}$ hr day, followed by artificial dusk (0·6 units) for 35 min per day. During the 4th and 5th weeks the numbers of attempted copulations which occurred during each 5 min of the period of exposure to artificial dusk were counted and the number of active males was recorded when the cages were observed at 5-min intervals, beginning $2\frac{1}{2}$ min after the flies were first exposed to artificial dusk. These observations were made on one cage selected at random each evening.

Figure 7 shows that even under constant dusk illuminance, the number of attempted copulations increased to a maximum and then decreased. Figure 8 shows that the number of active males also increased to a maximum and then decreased. It is probable therefore that two factors which influenced the time at which the sexual activity of the males ceases when the flies are under natural dusk are the onset of low illuminance or darkness, which had different effects on flies which had been kept under different day illuminances, and the time at which the flies begin sexual activity.

IV. Discussion

Only the results obtained from experiments done to investigate the effect of light during the day period on the age at which the male flies became sexually active are in need of further discussion.

Since young flies do not become sexually active and mature flies do so readily, it may be assumed that as male flies become older their sexual drive increases until maturity is reached. The specific outside stimulus for the release of this drive is exposure to dusk after exposure to a higher day illuminance. As flies fed on a protein hydrolysate develop mature testes and become sexually active earlier in life than those fed on the papaw mixture, it is apparent that internal drive is controlled by or at least correlated with the state of development of the testes, the rate of development of which is not affected by light. It is probable then that the relationship between sexual drive and age is not influenced by light. But the age at which males first become sexually active is affected by light. The effect of illuminance may be explained as follows: At optimal dusk illuminance the index of activity of males which had been under 240 or 60 lm/sq. ft is higher than the index of those which had been under 120 lm/sq. ft. This is true even when the flies are exposed 60 and 120 lm/sq. ft for 7½ hr only on isolated days and are kept under 240 lm/sq. ft for 7½ hr/day for the remainder of their adult lives. Dusk following either 60 or 240 lm/sq. ft is therefore a more effective stimulus for inducing mating behaviour than is dusk following 120 lm/sq. ft. Dusk following either 60 or 240 lm/sq. ft will be effective in stimulating flies to become sexually active when their internal drive is too low for dusk following 120 lm/sq. ft to be effective. Male flies will therefore become sexually active earlier in life when kept under either 60 or 240 lm/sq. ft for 7½ hr/day than when kept under 120 lm/sq. ft for the same daily period.



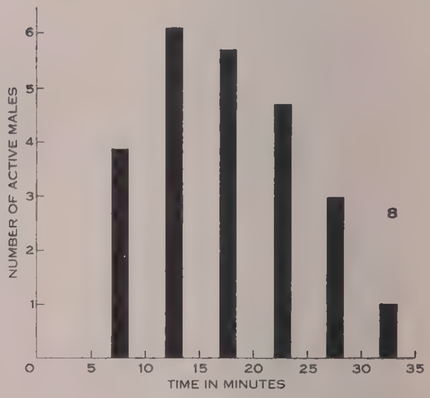


Fig. 7.—Number of attempted copulations occurring during each 5-min interval of exposure to artificial dusk.

Fig. 8.—Number of active males when observations were made at 5-min intervals during exposure to artificial dusk. The number of active males varied significantly with time ($P < 0{\cdot}01$) (periods 12½–32½ min inclusive considered).

The index of activity is controlled directly by the illuminance during the day period and not that just prior to dusk. The effect of light on feeding is not an important factor. The facts suggest that each day there is a build-up of internal drive and that the amount of this build-up depends upon the illuminance. This build-up of internal drive is probably due to the accumulation of some factor, perhaps a hormone, which is produced more rapidly in flies under either 60 or 240 lm/sq. ft than in flies under 120 lm/sq. ft. The difference in rate could well be a reflection of the activity of the central nervous system. (The general activity of flies under 60 or 240 lm/sq. ft is higher than that of flies under 120 lm/sq. ft.) When flies are not exposed to dusk on one day and are exposed to it on the next, they are no more active on the second day than when exposed to dusk every day. It is probable then that the accumulation of the factor was destroyed by a period of darkness.

No experiments were done to determine the indices of activity of flies under 240 lm/sq. ft for 2 or 4 hr/day, but the age at which males became active is affected by the daily period. This effect may perhaps be explained in a similar way to the effect on illuminance.

Several other species of trypetid have similar mating behaviour to that of *S. tryoni* in that they mate either exclusively or most frequently during dusk and in that the males emit a mating call.

Myers (1952) studied the mating behaviour of the solanum fruit fly, *S. cacuminata* (Hering) and found that it was very similar to that of *S. tryoni*. Other species which seem to behave similarly are *Dacus cucurbitae* (Coq) (Back and Pemberton 1917), *D. passiflorae* (Frogg.) (Simmonds 1936), and *Solanum halfordiae* (Tryon) and *S. bryoniae* (Tryon) which have been kept in this Laboratory. The mating behaviour of *D. dorsalis* (Hend.) differs from that of *S. tryoni* in that dusk is not necessary for mating. Flies kept either in constant light or constant darkness will mate, but when exposed to a high day illuminance followed by dusk they mate most frequently during the dusk period (Roan, Flitters, and Davis 1954). In *Anastrepha ludens* (Loew) mating occurs mainly in the late afternoon and early evening but has been observed at different times in the laboratory (Baker *et al.* 1944). No mention is made of calling behaviour by the males comparable with that observed in the other species.

It appears from this evidence that the type of mating behaviour found in *S. tryoni* is widespread among the Trypetidae, particularly the Dacinae. The adaptive value of such behaviour is that it increases the chance that a female has of mating. It ensures that all the sexually mature individuals in a population are sexually active at the same time and provides a mechanism whereby females are attracted to sexually mature males, even if only from a short distance.

V. ACKNOWLEDGMENTS

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I wish to thank Dr. L. C. Birch, Reader in Zoology, University of Sydney, for his guidance and encouragement during the course of the work. Thanks are also due to those at the University of Sydney and Division of Entomology, C.S.I.R.O., Canberra, who advised me during the preparation of this paper.

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AN INVESTIGATION OF THE LOW FREQUENCY OF MATING OF THE QUEENSLAND FRUIT FLY *STRUMETA TRYONI* (FROGG.)

By L. Barton Browne*

[*Manuscript received December* 19, 1956]

Summary

Unmated females of *Strumeta tryoni* (Frogg.) are attracted by the mating call of the male and accept attempts at copulation. Females which have recently mated are attracted by the calling but repel attempts at copulation. Females which have mated 7 days previously are not attracted by the calling and repel any attempts. One mating is sufficient to fertilize the eggs laid at least during the first 7 weeks of adult life.

I. Introduction

Barton Browne (1956) has shown that the mean frequency of mating in *Strumeta tryoni* (Frogg.) does not exceed 1·5 copulations per female in the first 7 weeks of adult life. This paper describes experiments to determine from the behaviour of mated females why repeated matings seldom occur and to find out whether one mating is sufficient for the fertilization of all the eggs laid by a female.

The methods for breeding the flies and controlling temperature, humidity, and light have deen described by Barton Browne (1956).

II. Results

(*a*) *Behaviour of Mated Females*

Batches of either eight unmated females, eight females which had mated 1 day previously, or eight females which had mated 7 days previously were placed in separate cages with eight mature males. The flies which were all in the 6th week of adult life were fed on papaw mixture (Myers 1952) and were kept under an illuminance of 240 lm/sq. ft for $7\frac{1}{2}$ hr/day, natural dusk for $\frac{1}{2}$ hr, and darkness for the remaining 16 hr each day. The numbers of attempted copulations of males with females and of males with other males were counted in the dusk periods (during which all mating activity occurred). These counts were made on 12 different evenings on a total of 12 cages, four for each treatment.

Table 1 shows that the ratio of the number of attempted copulations between male and male to the number of attempts between male and female was higher when the females had mated 7 days previously than when the females were unmated or had mated only 1 day previously. The total number of attempted copulations with either sex was lower when the females had mated 7 days previously. The females which had mated 7 days previously and those which had mated 1 day previously repelled attempts at copulation.

These results may be interpreted as follows: Unmated females were attracted by the mating call of the males (see Myers 1952) and accepted attempts at copulation.

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Females which had mated 1 day previously were still attracted to males but repelled attempts at copulation. Females which had mated 7 days previously were not attracted to males and repelled attempts at copulation. These findings explain why the females did not mate frequently. They also explain a finding reported in a previous paper (Barton Browne 1956) that eight different females usually participate in the first eight copulations occurring in a cage containing eight males and eight females.

TABLE 1

NUMBER OF ATTEMPTED COPULATIONS BETWEEN MALES AND FEMALES AND THE NUMBER BETWEEN MALES AND MALES WHEN FEMALES WERE UNMATED AND HAD MATED 1 AND 7 DAYS PREVIOUSLY

	Condition of Females at Commencement of Test		
	Unmated	Mated 1 Day Previously	Mated 7 Days Previously
Mean number of attempted copulations between males and males per cage per day	3·4*	3·1*	2·9*
Coefficient of variability (%)	26·5	25·0	29·6
Mean number of attempted copulations between males and females per cage per day	11·5	10·0	3·0**
Coefficient of variability (%)	20·7	25·0	36·6
Ratio of total number of male–male attempts to total number of male–female attempts	0·296	0·310	0·966

* No significant differences ($P > 0·05$).

** Differs significantly from the other two values ($P < 0·01$).

(*b*) *Influence of Repeated Matings on Fertility and Fecundity*

A cage containing a large number of flies fed on protein hydrolysate, sugar, and water was exposed to natural dusk each evening during the first 2 weeks of adult life. On each occasion all the pairs of copulating flies were removed to "Perspex" cages, eight pairs to each cage. In five of the cages the males were removed immediately after copulation had ceased; in another five, the males were allowed to remain. All the cages were exposed to an illuminance of 240 lm/sq. ft for 7½ hr and to natural dusk for 40 min at the end of the period of illumination each day. The number of eggs laid was counted and the percentage hatched from these determined until the flies were 7 weeks old, when the females were dissected and the spermathecae examined for the presence of sperm. After the transfer to "Perspex" cages some additional copulations occurred during the daily dusk periods in the series in which the males remained with the females. These occurred at an average rate of 0·45 copulations per female to the end of the 7th week making the total rate of copulation in this series 1·45 copulations per female over the experimental period. The rate in the series from which the males were removed was, of course, 1 copulation per female.

Table 2 shows that neither the number of eggs laid nor the fertility of the eggs was affected by the additional matings. In neither series of cages did the fertility decline with age. There were many active sperms in the spermathecae of all the females at the end of 7 weeks of adult life, i.e. after the fertilization of a mean of about 850 eggs. This probably exceeds the number laid by females in nature.

TABLE 2

COMPARISON OF EGG PRODUCTION AND FERTILITY RATE OF S. TRYONI EXPERIENCING 1 AND A MEAN OF 1·45 COPULATIONS PER FEMALE

The effect of the extra copulations is not significant ($P > 0{\cdot}05$)

Copulations per Female	Egg Production and Fertility Rate	Week of Life					
		3rd	4th	5th	6th	7th	
1·0 (males removed after copulation)	Mean eggs/female	158·2	183·8	183·6	172·2	161·8	859·6
	Coefficient of variability (%)	27·8	22·1	17·9	24·5	20·9	10·1
	Percentage hatch	92·4	95·4	90·8	92·6	93·1	92·1
1·45 (males remained with females throughout)	Mean eggs/female	172·6	172·8	191·8	163·6	170·4	871·2
	Coefficient of variability(%)	17·5	27·8	15·5	20·0	23·8	11·1
	Percentage hatch	91·2	96·0	89·6	94·2	92·6	92·3

III. DISCUSSION

The frequency of copulation of only a few species of insects is known, but the available evidence suggests that very frequent mating is unusual. Several insect species have been shown to behave like *S. tryoni* in that, although copulations in excess of 1 may occur, they are rare even under cage conditions where, by contrast with conditions in the field, the insects are closely confined. These include the tsetse fly *Glossina palpalis* (Rob.-Dev.) (Jackson 1949), the screw-worm fly *Callitroga americana* (Cush. & Patt.) (Bushland and Hopkins 1951), and the Australian sheep blowfly *Lucilia cuprina* (Wied.) (Barton Browne, unpublished data). It has been generally accepted that the social Hymenoptera mate only once (Allee *et al.* 1949, p. 422). More recent work (Taber 1954) has shown that multiple matings of the honey bee *Apis mellipera* L. are common and that each queen mates on the average $6\frac{1}{2}$ times. In the first mating flight which lasts only about 8 min the queens mate an average of 4 times. All the matings occur early in life and from the time the queen settles down in the hive she does not mate again.

Females of certain insect species mate after each oviposition. The walnut husk fly *Rhagoletis completa* Cress. (Boyce 1934), the Congo floor maggot *Auchmeromyia luteola* (F.) (Garrett-Jones 1951), and the grasshopper *Austroicetes cruciata* Sauss. (Andrewartha, personal communication) behave in this way.

Drosophila melanogaster females mate fairly frequently but rarely twice in quick succession (Rendel 1951). The Azuki bean weevil *Callosobruchus chinensis* (L.) mates at the high rate of 0·688 copulations/female/hr (Utida 1941). Other insects, the females of which are stated to mate frequently, are *Bruchus quadrimaculatus* F. (Brauer 1944), *Tribolium destructor* Uytt. (Reynolds 1944), and termites (Allee *et al.* 1949, p. 422).

Even less information is available on the number of copulations required by a female to ensure that there is no decline in fertility due to shortage of sperm. As in *S. tryoni*, one copulation is sufficient in the sheep blowfly *L. cuprina* (Mackerras 1936), the tsetse fly *G. palpalis* (Mellanby 1936), the beetle *Trogoderma versicolor* Creutz (Norris 1936), and the sheep ked *Melophagus ovinus* L. (Evans 1950). Some species of insects require repeated mating. In *Tetropium gabrieli* Weise, *T. fuscum* F. (Schimitschek 1929), *Tribolium destructor* (Reynolds 1944), *Anopheles punctulatus punctulatus* Donitz (Mackerras and Lemerle 1949), and *D. melanogaster* (Chiang and Hodson 1950) the fertility of eggs falls if the females do not mate at intervals. The fecundity of *T. gabrieli* and *T. fuscum* (Schimitschek 1929), *Tribolium confusum* Duv. (Park 1933), *B. quadrimaculatus* F. (Brauer 1944), and *Laemophloeus minutus* Oliv. (Davies 1949) is greater when males are left with the females, so that repeated mating can occur, than when the males are removed.

In sparse populations the frequency of meetings between males and females must often be very low. The chance of a female in a sparse population laying her maximum number of fertile eggs is greater if one mating is sufficient to fertilize all the eggs produced during her lifetime than if repeated matings are required. It is interesting to find that among those insects which require frequent copulation are some grain weevils which usually occupy fairly confined environments at high densities and *Drosophila* whose highly developed olfactory sense causes them to congregate near rotting materials and therefore have frequent contact. *S. tryoni*, *L. cuprina*, and *G. palpalis*, on the other hand, are insects in which congregations of males and females are less likely to form. It has been suggested by Evans (1950) that in *M. ovinus* the ability to lay a number of larvae after one mating is of advantage because transference from one host to another is entirely by contact. One mated female if transferred to another host is then capable of producing a heavy infestation.

IV. Acknowledgments

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UTILIZATION AND DIGESTION OF CARBOHYDRATES BY THE AUSTRALIAN SHEEP BLOWFLY *LUCILIA CUPRINA*

By L. G. Webber*

[*Manuscript received December* 3, 1956]

Summary

The nutritional value of 30 carbohydrates, mainly mono- and oligosaccharides and sugar alcohols, was determined by longevity experiments on the Australian sheep blowfly *Lucilia cuprina.*

Carbohydrates which maintain life for considerable periods are hydrolysed by enzymes present in the gut and salivary glands. The crop is an important site of hydrolysis of carbohydrates. The midgut and salivary glands secrete similar carbohydrases. These include two α-glucosidases, an α-galactosidase, and an amylase. A β-fructofuranosidase may be present, but this requires confirmation.

Field experiments indicated that the important natural sources of carbohydrates were the honeydew of coccids and psyllids and the nectar of *Eucalyptus* blossoms.

I. Introduction

It is known for vertebrate animals that carbohydrate is necessary as an energy source, and to ensure, amongst other things, adequate utilization of dietary protein (Munro 1951). For blowflies it has been known for some time that ingestion of a suitable protein in adequate quantity is essential for the maturation of eggs (Mackerras 1933; Evans 1935). Recently it has been shown that carbohydrate, as well as protein, is essential for the maturation of eggs in the Australian sheep blowfly *Lucilia cuprina* (Wied.) (Webber, unpublished data).

The availability and uptake of water, protein, and carbohydrate determines not only the longevity of *L. cuprina* but also its capacity to reproduce. It is clear, therefore, that females which mature eggs in the field must have had access not only to water and a suitable protein but also to some suitable carbohydrate.

Carbohydrates vary greatly in their capacity to maintain life. Information of this nature for a number of carbohydrates is available for *Calliphora erythrocephala* Meig. (Fraenkel 1940), *Drosophila melanogaster* Meig. (Hassett 1948), and for *Phormia regina* (Meig.) (Hassett, Dethier, and Gans 1950). However, no data are available for *L. cuprina*, and the ability of this species to utilize 31 carbohydrates and carbohydrate derivatives is now reported. Under natural conditions the most readily available sources of carbohydrate are probably the nectar of flowers, the honeydew produced by some insects, and the glucose (mainly derived from glycogen) present in carrion. It will be shown that sugars available from these sources can be readily utilized by adult *L. cuprina*.

II. Methods

(*a*) *Feeding Experiments*

Batches of newly emerged flies (50 females and 50 males), from larvae which were provided at all times with an abundant supply of liver, were kept in

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12 by 12 by 12 in. cages at a temperature of 25°C and a relative humidity of 40 per cent., and uniformly illuminated for 9 hr each day. Each cage was supplied with cotton-wool pads saturated with 0·1M carbohydrate solution, the pads being changed at intervals so that gross contamination by microorganisms did not occur. Dead flies were counted and removed twice daily for the first 3 days and subsequently at 24-hr intervals. The time for 50 per cent. mortality of flies was used as the basis of comparison of food value.

(b) *Digestion Experiments* in vitro

The salivary glands and midguts were removed from five newly emerged female flies, broken up by means of a small, sharp scalpel, and then incubated at 37°C with 0·3 ml of the solution of the di-, tri-, or polysaccharide to be tested. The carbohydrate was dissolved in water (pH 6·5) or in an acetate buffer (Walpole 1914) of pH 3·6 or 5·4. A Seitz filter was used to sterilize the solutions of sugars used in the digestion experiments. Toluene was added to prevent the growth of microorganisms. The solutions were analysed for sugars after 5, 10, 20, 30, 60, 120, 240, and 300 min incubation, and also at the end of 24 hr.

Aseptic flies were used for this work as experiments had shown that, over a period of time, the cotton-wool pads became contaminated by microorganisms capable of producing monosaccharides from oligosaccharides. These flies were obtained from larvae raised aseptically (Lennox 1939), allowed to pupate in sterile sand, and to emerge in sterile jars.

To determine whether carbohydrases were also secreted by the midgut it was necessary to work with flies deprived of their salivary secretion by the severing of their salivary ducts. In this operation the fly was anaesthetized and held on its back by one strip of plasticine across the body and another across the head. The latter held back the head exposing the intersegmental membrane between the head and thorax. A fine pin which had been sharpened and bent was used to make an incision through this membrane and the common salivary duct was cut near its point of discharge on the labium. The adults were then fed on sterile water for 24 hr in order to flush out from the gut any ingested saliva. The guts of the flies were then removed, macerated, and incubated with the carbohydrates as above.

(c) *Detection of Sugars*

The digestion products of flies fed on solutions of different sugars and the products of the incubation of salivary glands and midguts with sugars were identified by one-dimensional paper chromatography. Whatman No. 1 filter paper was used. Three different solvent systems were used to develop the chromatograms, viz. *n*-propanol–ethyl acetate–water (7 : 1 : 2 v/v) (Albon and Gross 1952), *n*-butanol–acetic acid–water (4 : 1 : 5 v/v) (Partridge 1948), and *n*-butanol–ethanol–acetone–water (5 : 4 : 3 : 2 v/v) (Gray and Fraenkel 1953). Ketose sugars were detected with a solution of α-naphthol (1 g) in ethanol (100 ml) containing phosphoric acid (10 ml, 90 per cent.) and aldose sugars with ammoniacal silver nitrate. The benzidine–trichloroacetic acid reagent as described by Bacon and Edelman (1951) was used as a non-specific spray reagent to detect sugars. A solution which contained

TABLE 1

COMPARISON OF THE HALF-LIFE OF L. CUPRINA WITH THAT OF C. ERYTHROCEPHALA, P. REGINA, AND D. MELANOGASTER ON A SERIES OF CARBOHYDRATES

Compound	50% Mortality (days)			
	L. cuprina	*C. erythrocephala* (Fraenkel 1940)	*P. regina* (Hassett *et al.* 1950)	*D. melanogaster* (Hassett 1948)
Water	2½	3½	2	2
Nothing	2	2½	1	1
Pentoses				
L (+)-Arabinose	2	3½*	3½	2
D (−)-Arabinose	2	—	2	3
L (−)-Fucose	4	—	2½	3
D (−)-Lyxose	—	—	3½	—
L (+)-Rhamnose	2½	3*	3½	2
D (−)-Ribose	5	—	4	4*
D (+)-Xylose	3½	5*	4½	3
Hexoses				
D-Glucose	12½	—	—	—
D-Fructose	15½	20*	14	18
D-Galactose	13	33*	17	9
D-Mannose	7½	27*	18	14
L-Sorbose	2½	3*	3½	3
Disaccharides				
Cellobiose	2½	3	3½	2
Lactose	3½	4	3½	3
Maltose	15	18	23	17
Melibiose	11½	35	6	12
Sucrose	13	30	16	24
Trehalose	9½	29	15	21
Trisaccharide				
Raffinose	9½	21	13	28
Polysaccharides				
Dextrin	4½	20	—	8
Glycogen 1%	2½	3	—	4
Glycogen 5%	9	—	—	—
Starch 1%	See text	—	—	—
Sugar alcohols				
Ribitol	2	—	—	4
Dulcitol	1½	3	3	2
Erythritol	3	3½	3	3
Pentaerythritol	—	—	2½	1
i-Inositol	4	3	3½	6
D-Mannitol	7	16	—	6
D-Sorbitol	11	47	15	5

Table 1 (*Continued*)

Compound	50% Mortality (days)			
	L. cuprina	*C. erythrocephala* (Fraenkel 1940)	*P. regina* (Hassett *et al.* 1950)	*D. melanogaster* (Hassett 1948)
Miscellaneous				
α-D-Galacturonic acid†	4			
D-Glucosamine hydrochloride	2½			

* No configuration given.

† Undergoes mutarotation in water, so both α and β configurations will be present.

authentic raffinose, melezitose, sucrose, glucose, and fructose was included on each chromatogram to act as a standard. To inactivate enzymes in the biological preparations 5 μl of 0·01M mercuric chloride were applied to the position on the paper where the test samples were to be placed.

III. Results and Discussion

(a) *Utilization of Carbohydrates by Adult Flies*

Flies deprived of food and water survive for 2 days (50 per cent. mortality). When water only is supplied they live for 2½ days. The survival times of flies fed on a series of carbohydrates are listed in Table 1.

The carbohydrates may be conveniently divided into four groups, as follows:

(1) Those on which survival is high (more than 11 days);

(2) Those which enable flies to live for 7–9 days;

(3) Those on which flies survive for a few days longer than on water alone (3–5 days); and

(4) Those which flies do not utilize at all, judged by the fact that survival is the same as on water alone.

There was no indication of a fifth group of carbohydrates displaying toxicity by reducing the survival period below 2½ days. Females had a slightly longer period of survival than males.

Group (1).—Within this group the order of nutritional effectiveness was D-fructose > maltose > sucrose = D-galactose > D-glucose > melibiose > D-sorbitol.

Group (2).—In this group the order of effectiveness was raffinose = trehalose > D-mannose > D-mannitol.

Group (3).—This group included D(−)-ribose > dextrin > L-fucose = *i*-inositol = α-D-galacturonic acid > D-xylose = lactose > erythritol.

Group (4).—Carbohydrates completely lacking in nutritional value to *L. cuprina* were: D(−)- and L(+)-arabinose, L(+)-rhamnose, L-sorbose, cellobiose, ribitol, dulcitol, and D-glucosamine hydrochloride.

Starch suspended in cold water did not support life for more than $2\frac{1}{2}$ days. However, starch dissolved in boiling water could apparently be attacked by the flies' digestive enzymes as it supported life for 12 days. Glycogen at 1 per cent. concentration had no nutritional value, but at 5 per cent. concentration 50 per cent. of flies survived for 9 days.

Table 1 shows that, broadly speaking, the carbohydrates studied have similar nutritional value whether fed to *L. cuprina*, *C. erythrocephala*, *P. regina*, or *D. melanogaster*. Thus members of groups (1) and (2) support life well for all species, although their rated order of effectiveness varies somewhat.

(*b*) *Analyses of Gut Contents after Feeding*

By feeding coloured sugar solutions to the flies it was observed that, when a fly commences to feed, food first passed simultaneously to both the crop and the midgut. When it reached the middle region of the midgut it was halted for about 15 min before it passed on; meanwhile the crop filled. Information on the nature and site of action of the fly's carbohydrases was obtained by chromatographic analysis of the contents of the crop and midgut at appropriate intervals after a carbohydrate meal. Flies were dissected at various times (ranging from 3 min to several hours) after feeding on aqueous solutions of the di- and trisaccharides that support life. In no instance could these oligosaccharides be detected in the midgut. This indicated that these carbohydrates were hydrolysed very rapidly after entering the midgut. When the crop contents were examined 3 min after a solution of sucrose had been ingested, only sucrose was found. However, after 10 min, sucrose, glucose, and fructose were identified. Similarly with maltose, both maltose and glucose were found in the crop shortly after feeding. It is clear, therefore, that the crop is an important site for the hydrolysis of sugars.

On some occasions no sugars were detected in the midgut although the crop contained the fed sugar and its products of hydrolysis. Regurgitation of midgut digestive juices into the foregut is reputed not to take place in adult blowflies (Graham-Smith 1934) and it is unlikely that the foregut itself is involved in the secretion of carbohydrases. It therefore appears probable that the enzymes responsible are derived from the saliva.

(*c*) *Carbohydrases of the Salivary Glands and the Midgut*

The results of a series of *in vitro* experiments in which carbohydrates were incubated with salivary glands and midgut homogenates are given in Table 2. In all instances identical results were obtained for both enzyme preparations. These results indicate that the same carbohydrases are present in both the salivary glands and midgut.

(*d*) *Specificity of* L. cuprina *Carbohydrases*

The experiments described above have given conclusive evidence for the presence in both salivary glands and midgut of *L. cuprina* of an enzyme capable of splitting sucrose. The problem of whether this reaction is due to the presence of a β-fructofuranosidase or an α-glucosidase, or whether both enzymes are present can be investigated by separation of the oligosaccharides formed by the action of fly gut preparations on sucrose. It is now well established that the hexose residue split from sucrose by the action of invertase is not transferred exclusively to water (as in a simple hydrolysis to glucose and fructose), but that other sugars present in the reaction mixture may act as acceptors. In this way a series of oligosaccharides may be built up, and the nature of these oligosaccharides is dependent on whether a fructose or a glucose residue is transferred by the enzyme. Several workers have concluded that the invertase of yeasts and moulds is a fructofuranosidase (Bacon and Edelman 1950; White 1952; Bacon and Bell 1953; Bealing 1953; Bealing and Bacon 1953; Bacon 1954; and Edelman 1954). On the other hand, the invertase of honey (White and Maher 1953*a*, 1953*b*), of the cottony-cushion scale *Icerya purchasi* Mask., the soft scale *Coccus hesperidum* L. (Wolf and Ewart 1955*a*), and probably of a number of other insects (Waterhouse 1957) is a glucosidase. Gray and Fraenkel (1953) detected a sugar of the structure glucose–glucose–fructose in the excreta of the blowfly *Phormia regina* when fed on a sucrose solution. This same sugar, glucosucrose, (and other members of the series produced by adding additional glucose units to the glucose end of the molecule) has been demonstrated in honey and in the honeydew of a number of aphids and coccids (Duspiva 1953, 1954; Gray and Fraenkel 1953, 1954; White and Maher 1953*b*; Wolf and Ewart 1955*a*, 1955*b*).

TABLE 2

ACTION OF SALIVARY GLAND AND MIDGUT EXTRACTS OF L. CUPRINA ON VARIOUS CARBOHYDRATES SHOWING THE BREAKDOWN PRODUCTS

Carbohydrate	pH	Concn. (M)	Time (hr)	Products
Sucrose	6·5	0·1	24	Glucose, fructose
Trehalose	6·5	0·1	24	Glucose
Maltose	6·5	0·1	0·5–24	Glucose
Turanose	6·5	0·1	24	Glucose, fructose
Melezitose	6·5	0·1	30	Melezitose, glucose, fructose, sucrose or turanose or both
Melezitose	6·5	0·04	24	Glucose, fructose, sucrose or turanose or both
Melibiose	6·5	0·1	24	Melibiose
Melibiose	5·4	0·033	24	Glucose, galactose
Melibiose	3·6	0·033	24	Melibiose
Raffinose	6·5	0·1	24	Raffinose
Raffinose	5·4	0·033	24	Galactose, glucose, fructose
Starch boiled	6·5	1%	24	Glucose, maltose(?) plus another
Glycogen	6·5	1% & 5%	24	Glycogen, glucose, maltose(?)

Fly gut homogenates were incubated with 10, 20, 30, and 40 per cent. unbuffered aqueous solutions of sucrose and the preparations analysed by paper chromatography. For periods of incubation of up to 240 min a maximum of five oligosaccharides were detected with the α-naphthol reagent (for ketoses) when the chromatograms were developed with *n*-propanol–ethyl acetate–water. These oligosaccharides had R_{sucrose} (R_S) values of 0·18, 0·26, 0·34, 0·52, and 0·72. Fructose was also detected. No oligosaccharides could be detected when the chromatograms were sprayed with ammoniacal silver nitrate for the detection of aldoses. However, glucose was detected.

In another series of experiments fly gut homogenates were incubated with 40 per cent. sucrose in an acetate buffer of pH 5·0. The products formed were detected on paper chromatograms developed with *n*-butanol–ethanol–acetone–water. Only two oligosaccharides were ever observed ($R_S = 0{\cdot}42$, and 0·63) when the papers were sprayed with benzidine–trichloroacetic acid. Fructomaltose has the same R_S value as melezitose (0·50) in this solvent (Gray and Fraenkel 1953) indicating the absence of this oligosaccharide in *L. cuprina*. Even when a brei of aseptic flies was incubated under sterile conditions with 15 per cent. and 40 per cent. sucrose solutions at pH 5·8 no oligosaccharide with an R_S value of 0·50 could be detected.

The fact that all the oligosaccharides formed are ketose sugars, which would presumably be more readily formed by the transfer of fructose residues, and the absence of any evidence to indicate the presence of fructomaltose (glucose–glucose–fructose), the most probable trisaccharide resulting from glucose transfer, suggests that a detailed examination is required to determine whether the invertase present is a fructosidase or a glucosidase. In all previous work the effect of microorganisms has not been rigidly excluded as in the present tests.

Although it is not possible to decide on available information whether or not an α-glucosidase is involved in the hydrolysis of sucrose, nevertheless an α-glucosidase (perhaps a specific maltase) must be present in the gut preparations because of the hydrolysis of maltose.

To summarize then, the above work indicates the presence of the following carbohydrases in the midgut and salivary glands of *L. cuprina*:

1. At least two α-glucosidases (one acting on maltose and melezitose and another, a trehalase, acting on trehalose).
2. An α-galactosidase (acting on melibiose and raffinose).
3. An amylase (hydrolysing starch and glycogen).

This may be compared with the earlier work of Wigglesworth (1929) who concluded that an amylase was the only carbohydrase secreted by the salivary glands of *C. erythrocephala*, whereas the midgut contained amylase, an invertase, and a maltase and Fraenkel (1940) who concluded that only an α-glucosidase and α-galactosidase were present in the gut of *C. erythrocephala*.

IV. Ecological Aspects of Fly Nutrition

It is reasonable to assume that, in general, the range of digestive enzymes possessed by an organism is correlated with the nature of its normal foodstuffs. It was, therefore, of interest to determine what carbohydrate sources were used as food by wild flies and whether the flies possessed the enzymes necessary to utilize these carbohydrates. The most important carbohydrate sources for wild flies would probably be carrion, the nectar of some flowers, and the honeydew excreted by psyllids and coccids.

Flies were fed on water and given access to flowers from 18 species of plants (Table 3). With the exception of those feeding on *Euonymus japonicus* flowers and eucalypt blossom, the flies died as rapidly as they would have on water alone. In one experiment 50 female and 50 male flies were released in a muslin net 9 by 6 by 4 ft covering a bed of asters. Water was provided. When sucrose was also provided in dishes, 40 flies were still alive on the 10th day. However, without the addition of sucrose, all flies died within 3 days, indicating that they were unable to obtain their carbohydrate requirements from these flowers. The inability of flies to survive on most species of flowers is presumably due to the inefficiency in gathering nectar of the short, less flexible proboscis of the fly compared with the long flexible proboscis of the honey bee.

TABLE 3

SPECIES OF PLANTS THE FLOWERS OF WHICH WERE OFFERED AS A SOURCE OF CARBOHYDRATE TO ADULT L. CUPRINA. ONLY THOSE MARKED WITH AN ASTERISK MAINTAINED LIFE

Common Name	Botanical Name	Common Name	Botanical Name
Aster	*Callistephus chinensis* Nees	Petunia	*Petunia hybrida*
Australian blackthorn	*Bursaria spinosa* Cav.	Red clover	*Trifolium pratense* L.
		Rose	*Rosa* sp.
Carrot	*Daucus carota* L.	Spear thistle	*Cirsium lanceolatum* L.
Convolvulus	*Convolvulus* sp.	Snapdragon	*Antirrhinum orontium* L.
Dandelion	*Taraxacum officinale* Weber	Tree tobacco	*Nicotiana glauca* Graham
Eucalypt	**Eucalyptus blakelyi* Maid.	White clover	*Trifolium repens* L.
	**E. melliodora* A. Cunn.	Wild mustard	*Brassica adpressa* Boiss.
	**E. macrorrhyncha* F. Muell.	Zinnia	*Zinnia* sp.
Fuchsia	*Eremophila maculata* F. Muell.	Japanese spindle tree	**Euonymus japonicus* L. (Celastraceae)
Marigold	*Calendula* sp.		

Flies were next fed on the honeydews excreted by two coccids (*Eriococcus coriaceus* Mask. and *Eriococcus* sp.) and a psyllid (*Spondyliaspis eucalypti* (Dob.)). *E. coriaceus* and *S. eucalypti* were feeding on *Eucalypus blakelyi*, and the second *Eriococcus* species was feeding on *E. macrorrhyncha*. The flies were still alive at the end of 10 days when the experiment was terminated, indicating that these honeydews are an important source of carbohydrates for wild flies.

In an attempt to determine where *L. cuprina* was obtaining its food in the field, wild flies were caught and their crops analysed immediately for carbohydrates. Possible sources of carbohydrate in the vicinity of the traps were also collected and offered to newly emerged caged flies. After about 24 hr the crops of these flies were analysed and the results compared with those obtained from wild flies. In one field where there were many young eucalypts (mainly *E. blakelyi*) it was found that the natural food was *E. coriaceus* honeydew. This honeydew contains raffinose, sucrose, fructose, glucose, glucose 1-phosphate, glucose 6-phosphate, and a carbohydrate, probably fructomaltose. In another field, which was typical of the open savannah country near Canberra, a few large eucalypts, mainly *E. melliodora*, spaced about one tree to 20 acres, were in blossom, the only other flowers being dandelions and spear thistles. Flies were unable to survive when fed on spear thistles or dandelions but lived for 10 days when fed on eucalypt blossom. The wild flies had sucrose, glucose, and fructose in their crops on capture, as did the laboratory-reared flies fed on eucalypt blossom. The crops of flies caught in Canberra contained abundant sucrose, glucose, and fructose and a little maltose, indicating that they were obtaining their carbohydrates from nectar.

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THE DISTRIBUTION OF THE EGGS OF MAMMALIAN LICE ON THEIR HOSTS

III. THE DISTRIBUTION OF THE EGGS OF DAMALINIA OVIS (L.) ON THE SHEEP

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Summary

The eggs of *Damalinia ovis* (L.) are attached to the wool and hair fibres of the sheep and have a vertical and lateral distribution, i.e. they may be laid at different distances from the skin and their distribution over the body of the sheep may vary.

The vertical distribution of the newly laid eggs of *D. ovis* is determined by the distance to which the temperature zone suitable for oviposition extends from the skin. This is regulated mainly by skin temperature, skin topography, the depth of the air blanket trapped within the fleece, and atmospheric temperature. Usually the eggs are laid within ¼ in. of the skin.

The factors which influence the lateral distribution are the presence of suitable fibres and temperatures for oviposition. Eggs are not laid on the bare areas of the body because fibres are absent. There are, however, few bare areas on the sheep. The bases of certain hairs on the face, legs, and axilla and inguinal regions are too large in diameter for oviposition but mingled with them are many fibres which are suitable. As there are no areas on the sheep which are entirely covered with hairs of unsuitable diameter for oviposition, fibre diameter has little influence on the lateral distribution of the eggs of *D. ovis*. When sheep are exposed to low atmospheric temperatures, the skin temperature of the extremities falls below that at which *D. ovis* can oviposit. Skin temperature is the main factor which determines the lateral distribution of the eggs of *D. ovis* on the sheep.

I. Introduction

In Part II of this series (Murray 1957*b*) it was shown that the major external stimuli which influenced the oviposition behaviour of *Damalinia ovis* (L.) were the physical features of the environment. The requirements are temperatures between 35 and 40°C, which enable stages 2 and 3 of the behaviour pattern to proceed satisfactorily, and the presence of a fibre of suitable diameter to supply a tactile stimulus just prior to egg laying in stage 3. The absence of either of these necessities can prevent oviposition (Murray 1957*b*).

The eggs of lice are distributed in two ways. They may be laid at different distances from the skin, hereafter referred to as the vertical distribution, and their distribution laterally over the body of the host may also differ.

This paper deals with the influence of variations of temperature and fibre diameter on the distribution of the eggs of *D. ovis* on the sheep.

II. Distribution of the Eggs in the Natural Environment

Several very heavily infested sheep were examined and eggs were found easily on the sides and back where the populations of lice were densest. Elsewhere, eggs

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were also found readily where lice were present, except on the ears and legs, and none was found on the bare areas of the body.

Because the eggs are attached to the wool fibres, they are gradually carried away from the skin as the fibres grow. It was necessary, therefore, to determine their age in order to locate the exact site of oviposition.

(*a*) *Technique for the Examination of a Louse Population*

The wool from areas of *c*. 1 sq. in. was dry-shaved from the skin with a sharp scalpel, care being taken not to disturb the arrangement of the fibres. This gave a neat staple of wool of which only a small vertical section, removed with sharp scissors, was examined at a time. The section of the staple was soaked in xylol and squeezed between two glass slides, the upper one of which was ruled with parallel lines at ¼-in. intervals so that the length of the staple and the position of the eggs could be determined. As xylol has about the same refractive index as wool fibres, the sample was rendered transparent which enabled the lice and eggs to be seen clearly. Furthermore, the living lice and eggs could be distinguished from the dead because the living appeared white and opaque but the dead were transparent. As the embryo could be seen within the egg it was possible to determine its stage of development. In addition, an egg which had hatched could be distinguished from an egg which had died because the egg cap of the dead egg was undisturbed. In some instances it was possible to determine the stage of development at the time of death; not infrequently death occurred during hatching.

When it was inconvenient to examine the material on the day of collection, it was stored in a deep-freeze chamber in which it could be kept in a satisfactory condition for several months.

(*b*) *Results*

The distribution of all recently laid eggs was determined in fleeces of different lengths. All the samples were collected in the winter months from sheep which were kept under cover. The results are presented in Figures 1(*a*) – 1(*e*) from which it may be seen that over 70 per cent. of the recently laid eggs in each sample were laid within ¼ in. of the skin and that, as the fleece became longer, they were found further from the skin.

III. THE INFLUENCE OF VARIATIONS IN HAIR DIAMETER IN THE ENVIRONMENT

The principal bare areas on the sheep are under the tail, around the vulva, the lips, inside the ears, and the hoofs. Elsewhere the body is covered with two types of fibres: the wool fibres, which cover most of the body, and the hair fibres which occur on the legs, the face, the axilla, and the inguinal regions. The hair fibres vary considerably in the diameter at their base and tend to taper towards the tip; although many are as fine as wool fibres the diameter of others may be as much as 10 times greater.

A collection of 900 lice was placed in a glass tube with hair from the leg of a sheep and kept at 37·5 C and 60 per cent. R.H. which are the optimum conditions for oviposition (Murray 1957*b*). After 48 hr 106 eggs had been attached to the fine hairs and two to the coarse hairs. Of the latter, one was attached to a tapering tip and the other to the base of a hair.

Two other collections of lice were exposed to the same conditions of temperature and humidity and hairs from the axilla were placed with one and hairs from the inguinal region with the other. On the hairs from the axilla region, 194 lice attached 21 eggs to the fine fibres, 21 to the thin ends of the coarse fibres, and four to the bases of the coarse fibres. On the hairs from the inguinal region, 191 lice attached 26 eggs to the fine fibres and 41 to the thin ends of the coarse fibres.

In two further experiments under the same conditions, fine fibres and the basal portions of coarse fibres, from which the tapered tips had been removed, were used. After 48 hr 630 lice had attached 45 eggs to the fine fibres whereas no eggs were attached by another 510 lice to the bases of the coarse fibres.

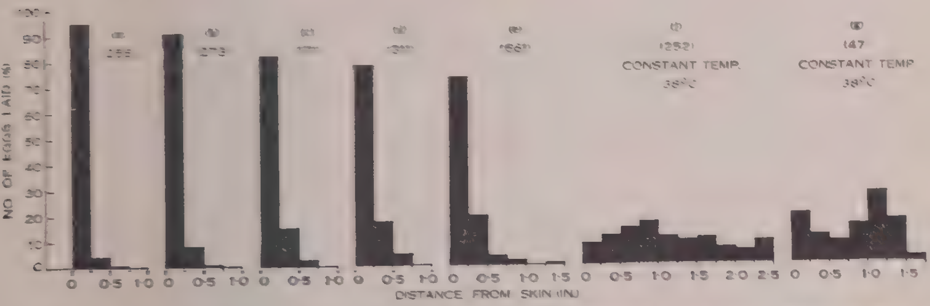

Fig. 1.—Vertical distribution of the eggs of D. ovis in the fleece of the sheep. Number of eggs examined in each fleece is shown in parenthesis on each figure. Fleece lengths are as follows: (a) 1½ in.; (b) 1½ in.; (c) 2½ in.; (d) 3 in.; (e) 4 in.; (f) 2½ in., temperature 38°C; (g) 1¾ in., temperature 38°C.

It can be concluded from these several experiments that the diameter of the base of some of the hair fibres from the legs, face, axilla, and inguinal regions of the sheep was too great to permit *D. ovis* to attach its eggs.

IV. The Influence of Variations in the Temperature of the Environment

(a) *The Distribution of the Eggs of* D. ovis *when a Constant Temperature was Maintained within the Fleece of a Sheep*

The temperature gradient in a sheep's fleece was eliminated by circulating water at a controlled temperature through a copper tank which was in contact with the tips of the fleece (see Fig. 2). A skin pedicle *A* which had been made on the side of the thorax of a sheep several months earlier was shaved except for the experimental area *B*. The experimental area was about 1½ by 3 in. in size. Around the pedicle was assembled a wooden framework *C* to support a copper tank *D* at the tip of the fleece of the experimental area. These two structures formed a cylinder around the pedicle and each end was sealed with foam rubber *E*. Thermocouples *F* were placed at different levels in the fleece of the experimental area. The whole apparatus was covered and secured by straps around the sheep. The temperature at the tip of the fleece was kept at 38±1°C so the temperature gradient within the fleece was eliminated.

Lice were collected daily and placed in the wool in this region. After a week the wool was shaved from the skin and the distribution of the eggs determined. The results are presented in Figures 1(*f*) and 1(*g*) from which it will be seen that eggs were laid throughout the whole length of the fleece.

The vertical distribution of the eggs is therefore determined by the distance to which the temperature zone suitable for oviposition extends from the skin.

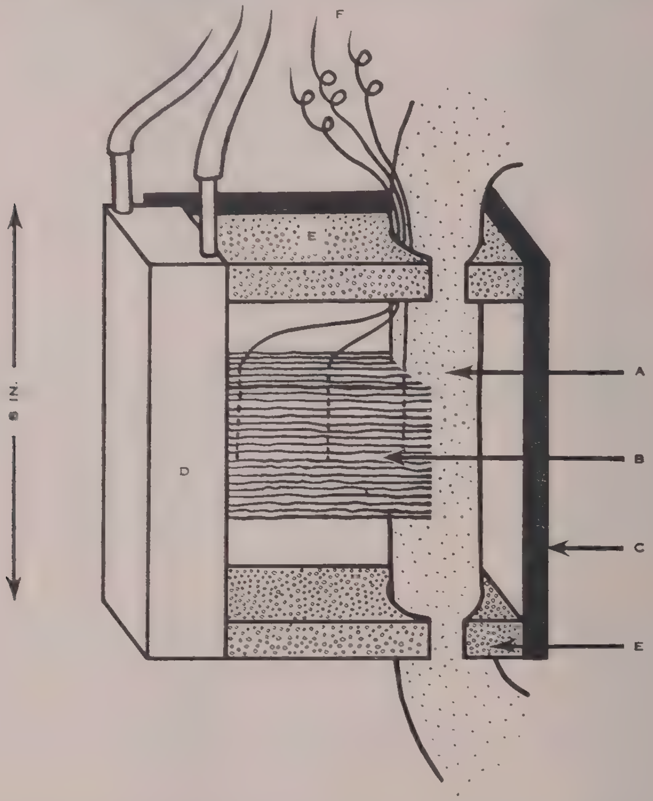

Fig. 2.—Apparatus used to eliminate the temperature gradient in the fleece of a sheep. *A*, skin pedicle; *B*, wool fibres of experimental area; *C*, wooden framework; *D*, copper tank; *E*, foam rubber; *F*, thermocouples.

(*b*) *Factors which Influence the Extent of the Temperature Zone Suitable for Oviposition*

(i) *Skin Temperature.*—Two sheep were placed in a cool room in which the temperature was 10°C. After 18 hr the skin temperatures on corresponding parts of the bodies of the sheep were recorded by means of an apparatus embodying a thermistor bridge with a D.C. amplifier. This apparatus was adjusted to record a large temperature range with an accuracy of ±0·5°C. The skin temperatures on the bodies of the sheep were 36·5 ± 1°C, but, as may be seen in Figure 3, the skin temperatures from the elbow joints to the feet, stifles to feet, root to tip of tail, bases to tips of ears, and around the nostrils were much lower; at the extremities of the limbs and tail the skin temperature approximated that of the atmosphere. When these sheep were placed in an atmospheric temperature of 30°C, however, the skin temperatures of these regions approximated that of the body.

(ii) *Skin Topography*.—Sheep of certain breeds, such as the Merino, possess numerous skin folds. When adjacent folds were in very close promixity it was found, by means of individual fine thermocouples, that constant temperature zones existed between them and thus increased the area of the 35–38°C temperature zone which favours oviposition.

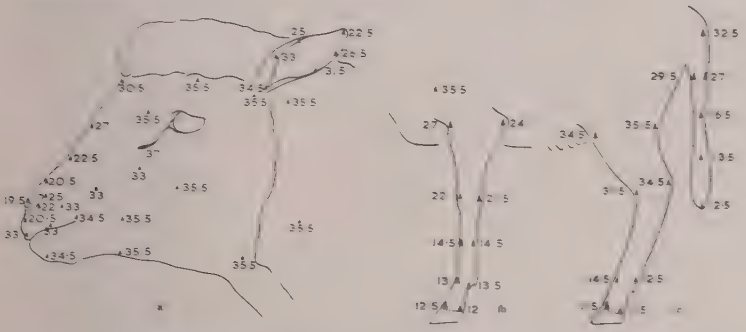

Fig. 3.—Skin temperatures (in °C) on various parts of a sheep when exposed to an atmospheric temperature of 10°C. (*a*) Head; (*b*) fore leg; (*c*) hind leg and tail.

(iii) *Thickness of the Air Blanket within the Fleece*.—Sheep with fleeces which maintained an air blanket from the skin to atmosphere of $\frac{1}{2}$, $1\frac{3}{4}$, $2\frac{1}{4}$, and 4 in. thick were housed in a climate room and exposed to constant temperatures of 17±0·5°C, 20±0·5°C, and 25±0·5°C for 18 hr before the temperature gradients within the fleece were recorded with either the thermistor bridge or thermocouple apparatus (Murray 1957*a*). Five areas over the body of each sheep were examined and the mean readings for each sheep when exposed to 20°C are shown in Figure 4. It may be seen that the temperature zone of 35–38°C increased in extent as the thickness of the air blanket increased.

The mean fleece-tip temperature of the 4-in. coat was 17°C in an atmosphere at 17°C, 21°C in an atmosphere at 20°C, and 25·5°C in one at 25°C. That of the $\frac{1}{2}$-in. fleece, however, was 22°C in an atmosphere at 17°C, 22·5°C in an atmosphere at 20°C, and 27·5°C in one at 25°C. Thus, the fleece-tip temperature of the shorter fleece was consistently higher than that of the longer fleece.

In another experiment, in which two sheep were used, the hair covering on the face was found to be $\frac{1}{8}$ in. deep and the mean temperature at the tips of the fibres at 11 positions on the face, when they were exposed to 24°C, was 30·2°C for one animal and 31·5°C for the other. Thus the rise in temperature at the tip was even more pronounced when the thickness of the air blanket was only of this depth.

(iv) *Atmospheric Temperature*.—In all the experiments described in Section IV(*b*)(iii) it was found that the fleece-tip temperature was always the lowest in the gradient and invariably approached that of the atmosphere. Consequently, the area of the 35–38°C zone increased as the atmospheric temperature rose.

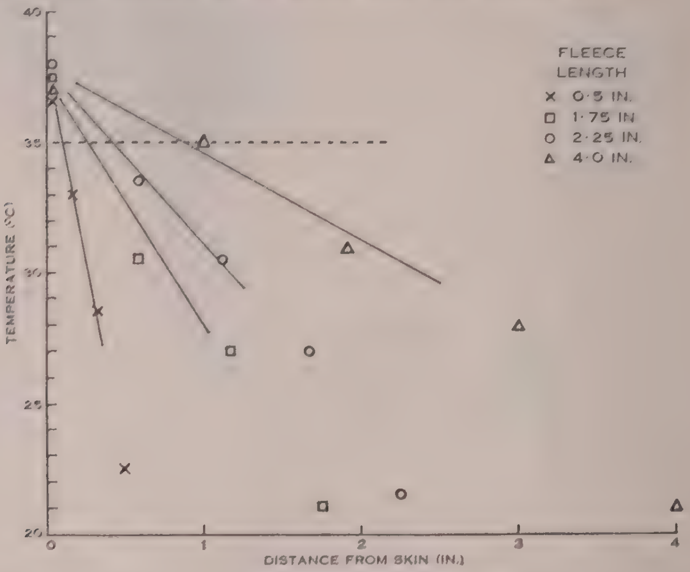

Fig. 4.—Temperature gradients within fleeces of different lengths at an atmospheric temperature of 20°C.

(v) *Solar Radiation.*—Sheep were exposed to solar radiation for 1 hr just before midday when the atmospheric temperature was 43–44°C. The fleece-tip temperatures recorded from various parts of the body of one of these sheep are presented in Figure 5. These temperatures are representative, and, as may be seen, the highest

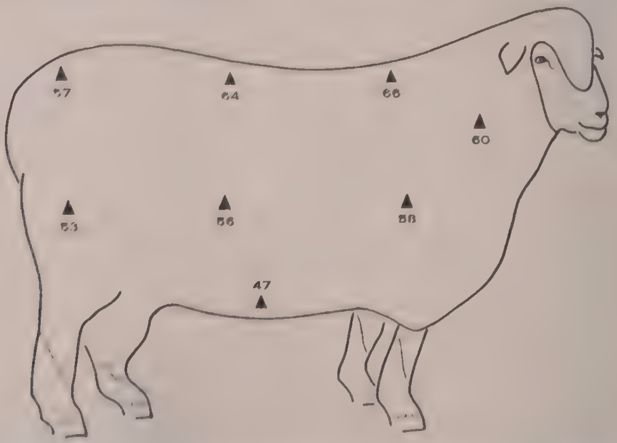

Fig. 5.—Fleece-tip temperatures (in °C) recorded on various parts of the sheep after a 1-hr exposure to solar radiation. The atmospheric temperature was 43–44°C.

temperatures were recorded on the back and progressively lower ones down the sides to the belly, which was sheltered from direct solar radiation. The highest temperature recorded on the back of any of the sheep was 71·5°C.

In Figure 6 are shown the temperature gradients which were recorded in the fleece on the back of the sheep referred to in Figure 5. One was obtained immediately after the 1-hr exposure to the sun, just before the sun was obscured by a cloud for 15 min; another was obtained after the sun had been obscured for 5 min, and the third gradient was recorded about 3 hr later during which period the sheep had been continuously exposed to the sun. It will be seen how rapidly the normal gradient can be re-established when the sun is obscured.

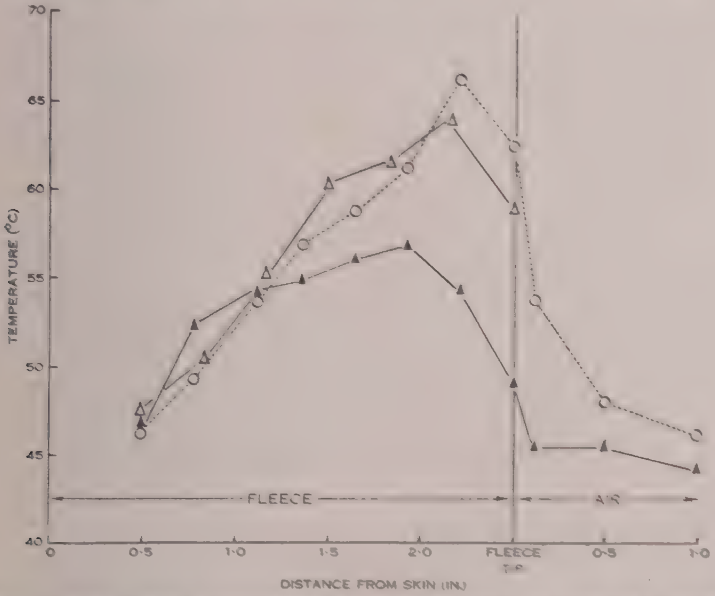

Fig. 6.—Temperature gradients recorded in the fleece of a sheep after exposure to solar radiation. The atmospheric temperature was 44°C (111°F). △ Immediately after an exposure of 1 hr just before midday. ▲ After exposure for 1 hr the sun was obscured by a cloud. This gradient was recorded 5 min later while the sun was still obscured. ○ Immediately after a further continuous exposure of 3 hr.

V. Discussion

Most of the eggs of *D. ovis* were found within ¼ in. of the skin but the thicker the fleece the greater was the zone in which eggs were found. It may be seen in Figure 4, that as fleece thickness increased so did the temperature zone of 35–38°C, which is suitable for oviposition (Murray 1957*b*). A rise in the atmospheric temperature had the same effect and when the temperature of the fleece tip was raised and maintained so that the temperature zone suitable for oviposition extended from skin to fleece tip, eggs were laid throughout the area. This distribution was similar to that obtained when lice were exposed to a constant temperature in a cell with suitable fibres (Murray 1957*b*).

The typical vertical distribution, therefore, is usually the result of an attraction to warmth in stage 1 of the behaviour pattern at the time of oviposition. This leads the louse to the skin where a temperature zone suitable for the completion of stages 2 and 3 is located. When the temperature gradient within the fleece is reversed, as can occur when sheep are exposed to the sun, suitable temperatures are again only to be found near the skin and the lice are driven into this zone by the high temperatures in the outer fleece.

Thus, the distance which the eggs of *D. ovis* may be found from the skin is determined by the extent of the temperature zone suitable for oviposition. Variations in the topography of the skin can increase this area. Merino sheep possess skin folds, which if prominent and in close proximity to one another create a constant temperature zone between them. Principally, however, it is determined by the temperature gradient which in turn is influenced mainly by the depth of the air blanket held between the fibres of the fleece and the temperature at the tip of the fleece. On many sheep the depth of the air blanket and the length of the fleece are the same but when the wool or hair fibres tend to lie parallel to the skin surface, as on the face and feet, the depth of the air blanket is the vertical distance from skin to atmosphere. The factors which influence the temperature of the tip are radiation from the skin, the thermal conductivity of the fibres, atmospheric temperature, and solar radiation. When the air blanket is greater than $\frac{1}{2}$ in. in depth, the two latter, in particular the atmospheric temperature, are more important. However, when the blanket is less than $\frac{1}{2}$ in., radiation from the skin and the thermal conductivity of the coat cause the temperature at the tip to become considerably greater than that of the atmosphere. A consequence of this is that even on the face and legs where the air blanket is thinnest, the depth of the suitable temperature zone next to the skin is adequate to accommodate a louse for oviposition. Thus, provided suitable fibres are present, and provided skin temperature approximates body temperature, *D. ovis* could oviposit anywhere on the body of sheep.

If no fibres are present, no eggs will be found, nor will eggs be laid if the part of the fibre which is in the suitable temperature zone is too great in diameter (Murray 1957*b*). No eggs are found on the bare areas of the sheep because fibres are absent. There are, however, few bare areas on the sheep. The bases of certain hair fibres which are plentiful on the legs, face, axilla, and inguinal region of sheep were shown to be too great in diameter, but mingled with these are many fibres which are of a suitable diameter for oviposition. Consequently, fibre diameter plays little part in determining the lateral distribution of this species on sheep. However, when sheep were exposed to an atmospheric temperature of 10°C, the skin temperature in certain situations on the extremities approached that of the atmosphere and the suitable temperature zone of oviposition next to the skin was eliminated. Skin temperature, therefore, determines the lateral distribution of the eggs.

The mechanisms which regulate the skin temperature of the extremities have been studied on the ears of rabbits (Grant 1930) and cattle (Beakley and Findlay 1955), and the presence of arterio-venous anastomoses has been demonstrated (Grant 1930; Findlay and Goodall 1953). The temperature to which an animal is exposed determines whether these anastomoses dilate or contract and thus decrease or increase the amount of blood circulating in the area distal to the anastomoses. Thus, the skin temperature of the extremities can vary according to the habitat of the animal, during the course of a day, and from one season to another.

There is little information on seasonal variation in the distribution of the eggs of *D. ovis* on the sheep but such variations are well known to occur with *Haematopinus eurysternus* (Nitz.) on cattle. During the summer months, breeding colonies of *H. eurysternus* become established on the ears of cattle (Craufurd-Benson 1941; Matthysse 1946; Brinck 1948). Beakley and Findlay (1955) have shown that, as the atmospheric temperature rises from 15 to 20°C, the skin temperature of the ears of cattle rises from *c.* 17 to *c.* 36°C and remains constant if the atmospheric temperature continues to be above 20°C. Atmospheric temperatures of this order are common during the summer months. Consequently, as the oviposition behaviour of *H. eurysternus* is basically similar to *D. ovis* (Murray 1957*a*), it is highly probable that oviposition by *H. eurysternus* on the ears can only occur when the temperatures are suitable. The establishment of breeding colonies of this louse on the tail and feet of cattle could be governed in a similar manner for it has been shown that at low atmospheric temperatures the skin temperature of these parts can drop to nearly that of the atmosphere (Thompson *et al.* 1954).

Low skin temperatures on the extremities have been reported from mammals as varied as man (Burton and Edholm 1955), reindeer, porcupine, ground squirrel, Stellar's sea lion, dog (Irving and Krog 1955), and the marsupials *Setonyx brachyurus* Quoy & Gaimard (Bartholomew 1956) and *Perameles nasuta* Geoffroy (Murray, unpublished data). It seems likely, therefore, that the skin temperature of the extremities of most mammals falls, in appropriate conditions, and thus governs the distribution of the eggs of many species of lice.

VI. Acknowledgments

I wish to thank Dr. I. W. McDonald for allowing me to use the climate rooms at the Sheep Biology Laboratory, Prospect, N.S.W., and Mr. W. Strauss who operated them for me. In addition I would like to thank Mr. L. Wilson of the same laboratory for the loan of the apparatus for determining skin temperatures.

To Mr. C. H. S. Dolling I am also grateful for providing facilities at the National Field Station, "Gilruth Plains", Cunnamulla, Qld., which enabled me to obtain measurements of temperature gradients in the fleeces of sheep when exposed to solar radiation.

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THE DISTRIBUTION OF THE EGGS OF MAMMALIAN LICE ON THEIR HOSTS

IV. THE DISTRIBUTION OF THE EGGS OF DAMALINIA EQUI (DENNY) AND HAEMATOPINUS ASINI (L.) ON THE HORSE

By M. D. Murray*

[*Manuscript received November* 27, 1956]

Summary

The eggs of *Damalinia equi* (Denny) and *Haematopinus asini* (L.) are attached to the hairs close to the skin with the end of attachment nearest to the skin.

The oviposition behaviour of *D. equi* is similar to that of *D. ovis* (L.). The only observable differences are that the gonopods are not used at the time of egg laying and the fibre is grasped by the claspers. Temperatures between 32 and 38°C are required for the maximum number of lice in a population to oviposit. Skin temperature can determine the lateral distribution of the eggs in the natural environment. A fibre of suitable diameter is also necessary. *D. equi* cannot attach its eggs to the coarse hairs of the face, mane, tail, and legs whereas *H. asini* is able to do so. As the coarse hairs of the mane, tail, and legs are not shed with the fine hairs of the coat at the beginning of summer and winter the shedding of the coat effects a more severe reduction of the population of *D. equi* than of *H. asini*.

I. Introduction

The two species of lice found on the horse are *Damalinia equi* (Denny) and *Haematopinus asini* (L.). *D. equi*, commonly known as the biting louse of the horse, is a mallophagan louse of the family Trichodectidae. *H. asini* is a blood-sucking louse of the order Anoplura. In general appearance *D. equi* is very similar to *D. ovis* (L.) of the sheep but only a few males have been found and the female is parthenogenetic. In this paper evidence is presented which shows that the oviposition behaviour of *D. equi* is similar to that of *D. ovis* and that the shedding of the fine hairs of the coat has an important effect in reducing the population of *D. equi* on horses.

II. Distribution of Eggs

The eggs of *D. equi* are attached to the hairs of the coat covering the body of the horse, but usually they are not found on the lower parts of the limbs, the tail, or the ears. An examination of 125 eggs showed that they were attached close to the skin and that the end of attachment was always nearest to the skin. Occasionally more than one egg was attached to the same fibre.

The horse possesses two types of hair covering: (i) the short fine hairs covering the body, upper parts of the limbs, the neck, and the head; and (ii) the long coarse hairs which give the poll, mane, tail, and lower parts of the limbs their characteristic

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appearance. A region of the mane was chosen for examination because it contained both hair types. Hair from this area was dry-shaved from the skin and the type of fibre to which each egg was attached was noted. A total of 103 eggs was examined and none was found to be attached to a mature coarse hair. In this same region eggs of *H. asini* were found attached to both types of hair.

III. The Oviposition Behaviour of D. equi

(*a*) *General*

The use of a warmed towel placed on the surface of the coat was found unsuitable for the collection of large numbers of these lice so the hair coat of a heavily infested area was dry-shaved from the skin with a sharp scalpel and the lice were subsequently separated.

Similar experimental cells and apparatus were used as in the study of the oviposition behaviour of *D. ovis* (Murray 1957*a*, 1957*b*).

(*b*) *Description of the Oviposition Behaviour*

Lice, together with glass wool on which they readily lay eggs, were placed in cells in which a temperature gradient from 18 to 36°C was maintained. It was observed that *c.* $\frac{1}{4}$–1 hr before laying an egg, the female moved to the warm end of the cell where it stayed until the egg was laid. During most of this period the louse remained with its head pointed in the direction of the warm end of the cell. Contractions within the abdomen were frequent; occasionally it was contracted to one side and then to the other, or the posterior tip was alternately contracted and extended. About 5 min before the egg was laid, the louse turned about, so that the posterior of the abdomen pointed towards the warm end. The louse backed towards the warm end and, after remaining motionless for about a minute, raised its gonopods away from the abdomen and grasped a fibre with the claspers situated on the posterior abdominal segment. The abdomen was rubbed up and down the fibre for several seconds and then the cement fixative was excreted suddenly. The surface of the cement set in *c.* 10 sec and *c.* 5 sec thereafter the louse began to expel the egg. The louse first expelled all the egg except the cap end and then assisted the completion of the operation by walking along the fibre. The expulsion of the egg occupied *c.* $\frac{1}{2}$ min and the louse then returned to the cooler end of the cell.

The oviposition behaviour of 25 lice was observed. All the eggs were laid at the warm end of the cell and all but one with the point of attachment nearest to the warm end.

(*c*) *Influence of a Fibre*

A collection of 126 lice was randomly divided into three equal groups which were placed in flat-bottomed glass tubes. In one of these there were no fibres, in another fine body hairs from a horse, and in the remaining tube coarse hair fibres from the mane. All lice were exposed to a temperature of 35°C at 60 per cent. R.H. This relative humidity was chosen because it has been shown that at high humidities the number of eggs laid by *D. ovis* is diminished (Murray 1957*b*). After 48 hr, three eggs had been laid in the tube with no hair, two in the tube with coarse hair fibres from the mane, and 22 in the tube with fine body hairs.

A fibre of suitable diameter had to be present, therefore, for oviposition to proceed satisfactorily.

(*d*) *Influence of Temperature*

A collection of 420 lice was divided randomly into six groups which were exposed to temperatures of 30, 32·5, 35, 37·5, 40, and 42°C respectively at 60 per cent. R.H. Glass wool was provided for oviposition. After 48 hr the number of eggs laid was counted. This experiment was repeated with another collection of 258 lice. The combined results are presented in Figure 1 in which it can be seen that the number of eggs laid increased with increase in temperature up to 37·5°C after which it declined until at 42°C no eggs were laid.

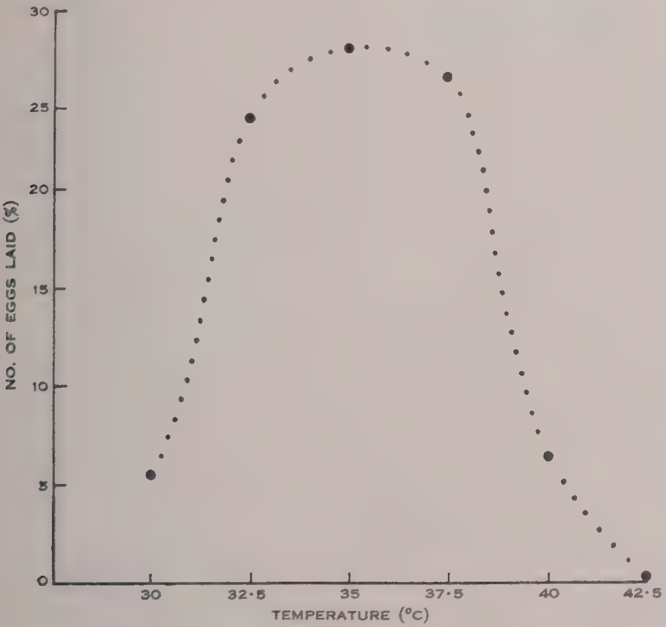


Fig. 1.—Influence of temperature on the number of eggs laid by *D. equi*.

From another collection, 24 lice were selected and each was placed in a separate glass tube without fibres. The length of the egg within each louse was measured. The lice were then divided randomly into two groups of equal numbers with a similar mean egg length. Oviposition was discouraged by withholding fibres. One group was exposed to a temperature of 30°C and the other to 35°C. After 24 hr the length of each egg within each louse was re-measured. The mean increase in egg length in those exposed to 30°C was 0·152 mm. and in those exposed to 35°C it was 0·156 mm. There was no statistically significant difference between the influence of these temperatures on the development of the egg within the louse.

IV. Discussion

The behaviour pattern of *D. equi* was similar to that of *D. ovis* (Murray 1957*a*). In stage 1 the louse moved to the warm end of a cell in which a temperature gradient was maintained and remained there until the egg was laid. In stage 2 it rested with its head pointed towards the warm end and in stage 3 it turned about and laid the egg (see Plate 1). The only observable difference was that the fibre was grasped by the claspers on the posterior segment by *D. equi*, whereas *D. ovis* held the fibre next to the abdomen by means of the gonopods. The rubbing of the abdomen on the fibre undoubtedly supplied a tactile stimulus which caused the secretion and excretion of the cement and thus initiated the expulsion of the egg.

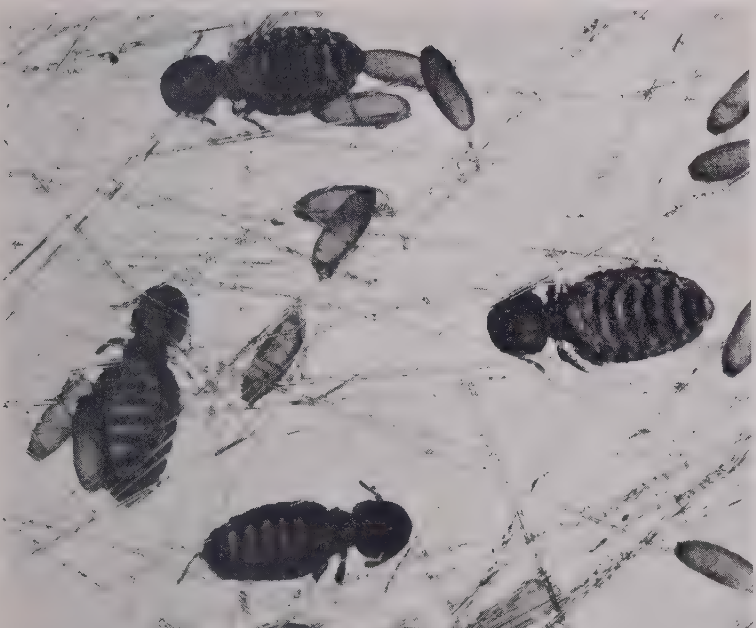
Temperature influenced the number of eggs laid and, as there was no difference in the influence of temperatures of 30 and 35°C on the development of the egg within the female, it was evidently a critical requirement for oviposition. Consequently any lowering of the skin temperature of the extremities of the horse below 30°C could influence the distribution of the eggs.

As with *D. ovis*, the presence of a fibre of suitable diameter is required for the normal completion of oviposition. *D. equi* cannot readily attach an egg to the coarse hairs of the mane. Thus, in regions such as the mane and tail, the distribution of the eggs can be determined by a preponderance of either of the two hair types. *H. asini*, the other louse found on the horse, is a larger louse and can attach its eggs to the coarse hairs. Consequently the distribution of its eggs is not determined by the diameter of the hairs.

Normally, the fine hairs of the horse are shed twice a year whereas the coarse hairs are not. Although this is a gradual process and the horse is never without a hair coat, shedding occurs at two definite periods: the beginning of summer and the beginning of winter (Duerden 1930). This results in the rapid loss of many of the hairs to which the eggs of *D. equi* are attached, and in one observation, when the winter coat was shed, 80 per cent. of the eggs of the population were lost. The shedding of the coat can thus affect the populations of these two species of lice on the horse differently.

A similar situation probably exists with cattle. Matthysse (1946) noted that *Damalinia bovis* (L.) invariably attached its eggs to the fine hairs of the coat which are normally shed by cattle in the spring (Duerden 1930). The eggs of *H. eurysternus* (Nitz.), however, can be found frequently on the tails of cattle where its eggs may be seen attached to the coarse hairs of the tip; these hairs are not shed each year (Duerden 1930). Consequently, the annual shedding of the coat may be expected to affect the *D. bovis* population more severely than that of *H. eurysternus*.

During the winter months, louse populations on horses, cattle, and many other animals increase in numbers and by the spring have frequently reached a density which, if there were no deterrent, could give rise to a great and rapid increase. Thus, any delay in the shedding of the winter coat could cause a great increase in populations of *D. equi* or *D. bovis*. In horses and cattle, the increasing hours of daylight in the spring appears to be an initial stimulus for the shedding of the coat (Burkhardt 1947; Yeates 1955) but it has been commonly observed in cattle that its effect may be delayed if the animals are in poor condition (Yeates 1955). This may well explain, DISTRIBUTION OF MAMMALIAN LICE EGGS ON THEIR HOSTS. IV



Damalinia equi laying eggs on glass wool. All eggs are aligned along the fibres with the end of attachment directed towards the warm end of the cell (bottom of plate). The first three lice from the left are in stage 2 and the first two from the right in stage 3 of the behaviour pattern. The sixth louse is not ovipositing.

in part, the clinical observation that animals in poor condition tend to harbour more lice than those in good condition.

On many mammals the type of hair varies from one part of the body to another. As variations in the shedding of different hair types are known to occur and as it is probable that fibre diameter is of importance in the oviposition behaviour of many mammalian lice, similar relationships to those described here in regard to two species affecting the horse are to be expected with other species.

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OBSERVATIONS ON THE LIFE HISTORY OF THE CAT LUNGWORM, *AELUROSTRONGYLUS ABSTRUSUS* (RAILLIET, 1898) (NEMATODA: METASTRONGYLIDAE)

By M. JOSEPHINE MACKERRAS*

[*Manuscript received February* 27, 1957]

Summary

First-stage larvae of *Aelurostrongylus abstrusus* (Railliet) developed readily in the garden slug, *Agriolimax laevis* (Muller), two moults occurring in this host.

When infected slugs were eaten by a cat, the third-stage larvae migrated at once to the lungs, where they underwent the third and fourth moults. The prepatent period in the cat was about 39 days.

When infected slugs were fed to laboratory-bred mice, the third-stage larvae were not digested, but congregated in great numbers in the gastro-splenic ligament, forming minute yellowish cysts. They were also recovered in small numbers from other parts of the body. Third-stage larvae survived unchanged in mice for 12 weeks, and probably would survive for longer periods. Mice were regarded as important auxillary hosts.

The minute adult worms were found in the bronchioles of the cat. They were always very difficult to locate. The eggs were laid in the alveoli, forming a characteristic pattern of small, solid nodules scattered throughout the lung tissue.

I. INTRODUCTION

Several helminthologists have already studied the lungworm of domestic cats in various parts of the world, but their findings have not always been in agreement. Cameron (1926, 1927*a*, 1927*b*, 1928, 1929) considered that mice were indispensable intermediate hosts, but Hobmaier and Hobmaier (1935*a*, 1935*b*) and Gerichter (1949) proved conclusively that land molluscs were the true intermediate hosts. The Hobmaiers also showed that mice and many other small vertebrates could act as auxillary or storage hosts.

Gordon (1933) was the first to record the presence of *Aelurostrongylus abstrusus* (Railliet) in Australia, basing his identification on the characters of the larvae in the faeces of a cat.

The present study was undertaken primarily to find out whether the infective larvae of this species invaded the central nervous system, as the rat lungworm had been found to do (Mackerras and Sandars 1955).

II. MATERIALS AND METHODS

Some cat faeces containing lungworm larvae were obtained by the courtesy of Mr. A. J. Bearup, School of Public Health and Tropical Medicine, Sydney. Infection was set up in the small, dark, garden slug, *Agriolimax laevis* (Muller), which proved to be a good intermediate host, third-stage larvae being obtained in about 3 weeks in the winter. To extract these, the slug was anaesthetized, the foot stripped off, teased

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up a little with needles, and placed on a muslin plug in a centrifuge tube filled with sterile tap water. After a few hours at 37°C, many larvae were found in the water, and could be concentrated by spinning.

Suckling kittens were used while still entirely dependent on the mother, as they would certainly not have been infected by eating slugs or mice at this stage. The kittens were either fed the foot of an infected slug, or the third-stage larvae extracted from slugs were given to them in milk with a pipette.

Laboratory-bred mice were used when about 3 or 4 weeks old. In some experiments, the feet of slugs containing third-stage larvae were fed to them; in others, first-stage larvae were extracted from the faeces of a cat by a modified Baermann technique, concentrated by spinning, and fed to the mice in water mixed with food.

III. AELUROSTRONGYLUS ABSTRUSUS

(*a*) *Adult Worms*

The adults obtained from experimentally infected cats were minute, thin, delicate worms; males 5·0–6·25 mm in length, average 5·2 mm (eight measured), by 0·07 mm in breadth; females 9·0–10·2 mm, average 9·3 mm (10 measured), by 0·1 mm. They conformed fairly closely to the descriptions by other workers. Oesophagus short, club-shaped, about 0·25 mm long (Plate 1, Fig. 3). In the female the two uteri lay parallel before uniting to form the common uterus. No sphincters were detected. The anus was 0·026 and the vulva 0·076 mm from the tip of the tail (Plate 1, Fig. 4). In the male the spicules were equal, curved, striated rods, 0·12–0·13 mm long. The gubernaculum had a raised central part with a channel on either side of it (Plate 1, Figs. 5 and 6).

Several observers have commented on the variability of the dorsal ray of the male bursa. Mueller's original figure, reproduced by Cameron (1926, 1929), shows two dorsal rays, quite widely separated at the base, and each bifurcating near its distal end. Subsequent observers figured the dorsal ray as a single, relatively broad structure, variously surmounted with papillary processes, and this appearance was seen in some of the worms obtained here (Fig. 5). However, in one specimen there was a series of four, very tiny, asymmetrically placed rays between the externo-dorsals of each side (Plate 1, Fig. 6).

(*b*) *Egg*

The egg is a very delicate structure, with a thin, clear shell which is readily deformed. All stages of segmentation from about eight-cell to fully-formed larva were seen in scrapings from a nodule. Some of the earliest stages gave the following average measurements: shell 75 by 60 μ, range 84 by 66 to 63 by 54 μ; morula 50 by 40 μ, range 60 by 42 to 48 by 30 μ (five measured). The developing embryo occupies more and more space until it fills the egg shell.

(*c*) *Life Cycle*

First-stage larvae from the faeces of a cat measured 0·36–0·4 mm in length by 0·018–0·02 mm in maximum width. These measurements were made on unfixed material immobilized by heat. The oesophagus measured about 0·15 mm, and was provided with median and posterior bulbs, both weakly developed. The larvae adopted a characteristic J-shaped pose with the tail curved ventrally; they were easily recognizable by the peculiar double-notched tail (Fig. 1).

(i) *Development in the Intermediate Host.*—Development in the slug, *Agriolimax laevis*, was very similar to that described by Gerichter (1949) in other land molluscs. The first moult occurred about the 6th day, and the second about 3 days later, when the temperature ranged from 22 to 33°C. These periods progressively lengthened as the temperature fell.

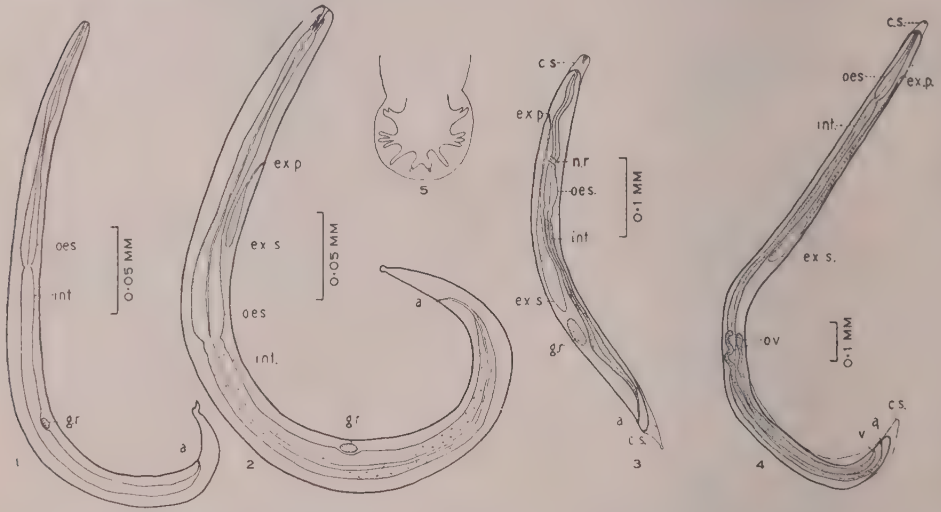

Fig. 1.—First-stage larva from faeces of a cat. Fig. 2.—Third-stage larva from foot of slug. Fig. 3.—Third-stage larva from lung, in process of third moult. Fig. 4.—Fourth-stage female larva from lung, in process of fourth moult. Fig. 5.—Ventral view of bursa of male (diagrammatic). Figs. 1, 2, and 5 are drawn to the same scale. *a*, Anus; *c.s.*, cast skin; *ex.p.*, excretory pore; *ex.s.*, excretory sac; *g.r.*, genital rudiment; *int.*, intestine; *n.r.*, nerve ring; *oes.*, oesophagus; *ov.*, ovaries; *v*, vulva.

The third-stage infective larvae measured from 0·52 to 0·53 mm in length by 0·03 mm in width. The oesophagus measured 0·2 mm, the anterior part being long and narrow, and the posterior part club-shaped. There were chitinous thickenings with knob-like anterior ends in the anterior part of the oesophagus. Tail 0·038 mm, ending in a very characteristic rounded knob (Fig. 2).

(ii) *Development in the Cat.*—A kitten was fed infective larvae on 2 successive days, and killed and dissected 4 days after the second dose. Third- and fourth-stage larvae were found in the lung, and one larva was seen in the process of the third moult (Fig. 3). The largest fourth-stage larva found measured 0·88 by 0·045 mm. The oesophagus was without chitinous thickenings, and the tail had lost its little rounded knob.

A kitten was killed 10 days after infection, and fourth-stage larvae were found in the lung. One female larva, 2 mm in length, was seen in the process of the fourth moult. Another kitten killed after the same interval also yielded a fourth-stage female larva in the process of the fourth moult (Fig. 4). These observations are in close agreement with those of Hobmaier and Hobmaier (1935*b*).

In a kitten killed on the 17th day, young adults were found in the lung. One male measured 2·8 mm, and the females were a little longer. Egg production had not begun.

In a kitten killed on the 28th day, adults were found, usually in pairs, in the finer bronchioles. Egg laying had begun, scanty eggs, all in very early stages of cleavage, being found rather scattered in the lung.

In a kitten killed on the 32nd day, eggs were numerous, but still in early stages of cleavage. No larvae were found in the lung or in the faeces. The shortest periods observed between infection and the appearance of first-stage larvae in the faeces were 37 days (one kitten) and 39 days (two kittens).

In a kitten killed on the 41st day, the lungs were extensively mottled with clear, pale areas containing numerous eggs and larvae. The adults were found in the lung substance, usually in groups of two or three, as in Plate 1, Fig. 1. They were about the same size as at 32 days. Numerous larvae were present in the faeces.

In kittens killed during the first 4 weeks, other organs besides the lungs were searched for developing larvae, both directly by microscopic examination of snippings and squashes, and also after peptic digestion at 37°C overnight. These organs included brain, spinal cord, diaphragm, liver, mediastinal and mesenteric lymph nodes, omentum, and mesentery. Larvae were found on two occasions, once in a kitten killed 10 days after infection, and once in a kitten killed 8 days after the last dose and 11 days after the first dose of infective larvae. In both, larvae were found encysted in the omentum, and in one a single larva was also found in the diaphragm.

These larvae were still in the third-stage, and they seemed to be behaving in the way larvae do in the mouse, namely, lying-up indefinitely in the omentum. One can only speculate whether they might be liberated from encystment at some stage, and proceed to full development in the same host, or whether they would need to be eaten by another cat before proceeding any further in the life cycle.

IV. The Role of the Mouse in the Life Cycle

A mouse, which had been fed infected slug material, was killed on the 13th day. Large numbers of third-stage larvae were found encysted in the omentum. They occurred singly, or in little groups in pedunculated pockets, particularly at the edges of fat depots in the gastro-splenic ligament (Plate 1, Fig. 2). They were infective to a kitten. They were not detected elsewhere in the mouse during dissection, but, after peptic digestion, 12 were recovered from the small intestine with its mesentery and glands, and six from the carcass after the skin and viscera had been removed.

Three mice were kept for 12 weeks after feeding on infected slug material. Upon dissection, they presented the same picture as that described above, namely, a great concentration of unchanged infective-stage larvae in the gastro-splenic ligament. These larvae appeared normal in all respects, and there was no sign of reaction to their presence on the part of the host. They proved to be infective to a kitten.

First-stage larvae from the faeces of a cat were concentrated by the Baermann technique, and fed to two young mice in water mixed with their food. These mice were dissected 3 and 5 weeks later, but no larvae were found either directly or after peptic digestion.

It seems clear then that mice may act as important auxiliary or storage hosts. They are more acceptable to cats as food than are slugs. The experimental kittens almost invariably vomited repeatedly after ingesting infective larvae from slugs. It made no difference whether the larvae were still in the slug, or were swallowed in milk. Vomiting usually began within 5 min. After eating mouse omentum containing larvae, one kitten did not vomit at all, and a second did not vomit until nearly an hour after ingesting the material. The vomiting seemed to be due to irritation of the gastric mucosa by the larvae. Possibly mouse tissue is digested rather slowly, so that the liberation of larvae is more gradual, and irritation of the mucosa is reduced.

Laboratory-bred white rats also acted as storage hosts. Unchanged infective stage larvae were found encysted in the gastro-splenic ligament in two rats killed 14 days after ingesting infective slug material. One rat contained a few larvae, and the other at least 18 larvae. Rats may not be as favourable hosts as mice.

V. The Lesions in the Lung

All lobes have been found infected, the extent of damage being proportional to the number of worms present, and the appearance of the organ varying with the stage of the infection.

Heavily infected lobes were extensively mottled with pale nodules, which were about 1–2 mm in diameter, and translucent or pale creamy in colour in the early stages. Later, larger nodules were formed by the confluence of smaller ones. They formed an irregular pattern of raised, solid, creamy yellow areas on the pleural surface, the intervening areas appearing normal to the naked eye. The nodules were not confined to the pleural surface, but were scattered throughout the whole depth of the organ (Plate 2, Fig. 1).

The adult worms were found in little groups, usually a pair, sometimes one female with several males, and occasionally two or more females. They appeared to be in the terminal and respiratory bronchioles and alveolar ducts, and could with care be extracted without damage. The abundance of nodules and the scarcity of adults gave the impression that the worms moved about continually, the female laying a mass of eggs at intervals.

The ova appeared to be forced into the alveolar ducts, filling and distending them and the adjacent alveoli. An indication of this lobulated arrangement may be seen in Plate 2, Figures 2 and 3. The ova around the periphery usually developed first, and it is suggested that the development of those more centrally placed may be inhibited by a relative anoxia caused by the pressure of the surrounding ova. As this pressure is relieved by the departure of larvae, air can enter, and development of more ova is stimulated.

In sections from a portion of one lung of a kitten killed on the 32nd day, ova were scattered rather uniformly in the alveoli of a portion of one lobe (Plate 2, Fig. 4).

The reaction of the lung to the foreign bodies was a swelling of the alveolar walls, and a shedding of alveolar phagocytes and leucocytes into the respiratory tract. As each ovum developed into a larva, which left the alveolus and ascended the bronchioles, the space left became filled with these cells (Plate 2, Fig. 4). Around the margins of the resolving nodules there were areas of collapse and beyond this areas of emphysema. Giant cells were present in the resolving lesions in some sections.

VI. Duration of Infection

The course of the infection was followed at intervals in several kittens by extracting their faeces in a modified Baermann apparatus. First-stage larvae could always be found in moderate numbers by the end of the 6th week. The output then increased rapidly, and, in heavily infected kittens, enormous numbers of larvae were excreted during the 3rd to the 7th month. Their number then began to diminish. The infection was observed to die out in one lightly infected kitten by the 6th month. In two other animals it persisted for 8 and 9 months respectively.

VII. Discussion

Cameron studied the lungworm of cats in Britain, and came to the conclusion that mice were essential intermediate hosts. He based his conclusions on experiments, in which he fed first-stage larvae from lung or alimentary tract to tame white mice. Later he recovered larvae from the subcutaneous tissue of mice* and set up infections in cats by feeding experimental mice to them. This cycle was repeated, and the essential role of the mouse seemed to be firmly established.

A few years later, the Hobmaiers studied the lungworm of domestic cats in California. They failed repeatedly to confirm Cameron's findings, but succeeded in infecting land molluscs, and in transmitting the parasite to cats by feeding them infected molluscs. They also found that mice, sparrows, ducklings and chickens, frogs, toads, snakes, and lizards could act as auxiliary hosts (Hobmaier and Hobmaier 1935*b*).

Gerichter (1949) studied the lungworm of domestic cats in Palestine, and confirmed the Hobmaiers' findings that land molluscs were essential intermediate hosts.

Cameron (1928) observed that "the eggs in varying stages of development were evenly distributed throughout the lung and not isolated in any particular area." He inferred from this that they had been deposited in the blood stream. He was successful "in several cases" in finding adults in the pulmonary vessels (presumably in the arteries). However, other workers have failed repeatedly to confirm these observations, so that the occurrence of *A. abstrusus* in blood vessels must be exceptional. In the present series, sections of the lungs of five cats all showed the ova for the most part concentrated in nodules. Only in a sixth animal, killed before the infection, became patent, was there any suggestion that the eggs might have been blood-borne. As mentioned above, the eggs were scattered thinly over a portion of one lobe. The appearance, however, differed considerably from that seen in the lungs of rats infected with *Angiostrongylus cantonensis* (Chen), which normally lives in the pulmonary arteries. In the latter infection the grouping of ova clearly follows the distribution of the terminal arterioles (Mackerras and Sandars 1955, Plate 5, Figs. 3 and 4).

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* The larva which Cameron described and figured from the mouse appears very like a third-stage larva of *A. abstrusus*, except that it is much longer (0·725 mm) than any observed here. Perhaps this was a transcription error for 0·525 mm.

Two alternatives suggest themselves: either that Cameron had a mixed infection, one species inhabiting the pulmonary arteries and developing in mice, and the other being *A. abstrusus*; or that his experimental mice were already infected when he obtained them. They were all from "the same source", and might possibly have been fed on fresh vegetable material, and (accidentally) slugs or snails, from a garden inhabited by an infected cat. The second alternative seems more likely; but this does not explain why the adults were in blood vessels in his animals, and in the lung substance in those of other workers.

The identity, or otherwise, of the worms studied by observers in different parts of the world (Britain, California, Palestine, and Australia) requires investigation. There may be more than one species involved. Indeed, Gerichter found a second, rare species in Palestinian cats, which he named *Anafilaroides rostratus*.

However, the descriptions given by various workers agree fairly well with one another, and with the worms found in experimental cats in Australia. Some points of difference may be noted. Cameron (1927*a*) stated that the spicules were 0·075 mm long. Other workers have found them considerably longer: Hobmaier and Hobmaier (1935*b*) 0·13–0·15 mm, Gerichter (1949) 0·13–0·22 mm, present series 0·12–0·13 mm. Spicule length, however, is not a good character on which to base specific differences. In the rat lungworm, two races are known to occur in Australia, the typical race with spicules 1·1 mm long, and the southern Queensland race, in which they are only 0·49 mm long. It seems, then, that the difference in spicule length may be ignored.

The Hobmaiers found that the discharge of first-stage larvae ceased after 8 weeks, and that adults were still present after the discharge had ceased. In the present series, larvae were found in the faeces of experimental kittens for more than 7 months after patency, and adults, as well as numerous eggs and larvae, were present in a kitten killed 5 months after infection and 3½ months after patency. Development in *Agriolimax laevis* has been more rapid than has been reported for other molluscs. The minimum periods here in midsummer were: first-stage 6 days, second-stage 3 days. Gerichter recorded 11 and 4 days, and the Hobmaiers 10 and 28–35 days respectively. These differences are probably due to temperature. However, there is a possibility that different races are evolving in groups of cats now completely isolated from one another in different continents. Some differences in morphology and behaviour might be explained in this way.

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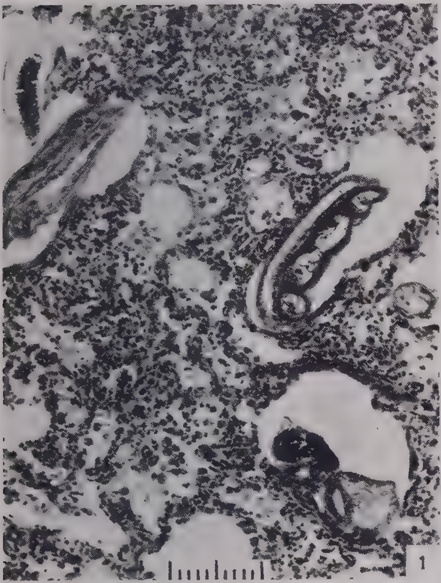
LIFE HISTORY OF THE CAT LUNGWORM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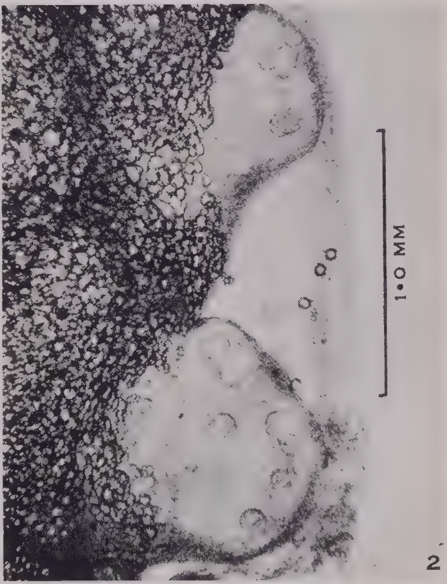

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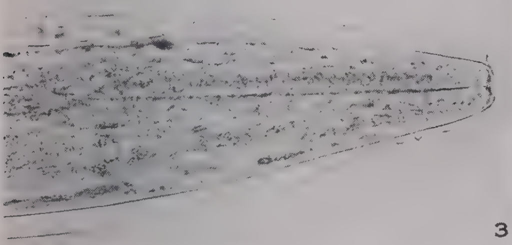
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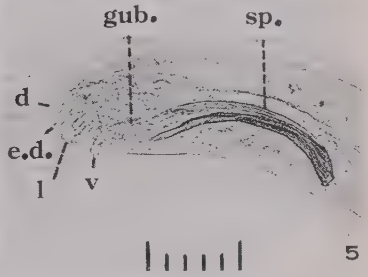

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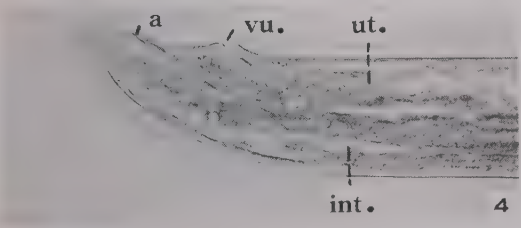

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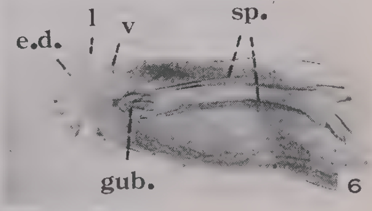

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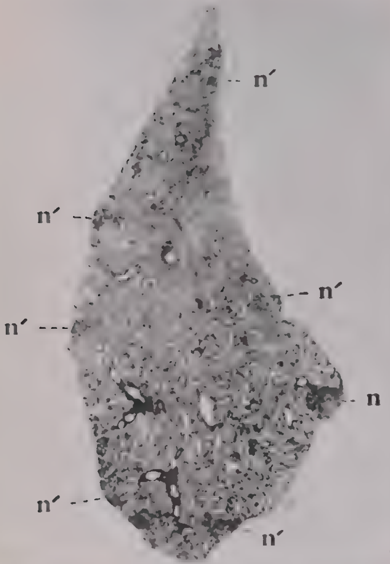

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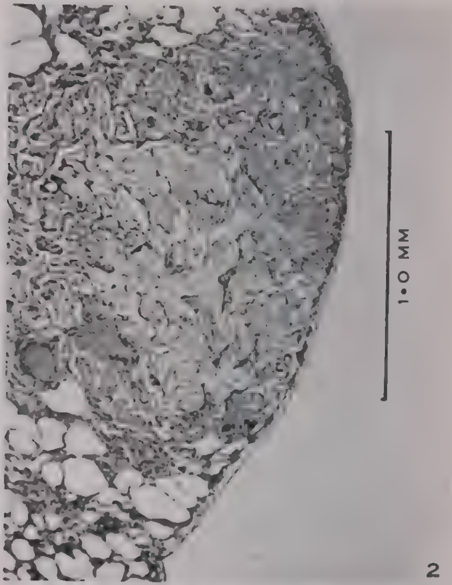

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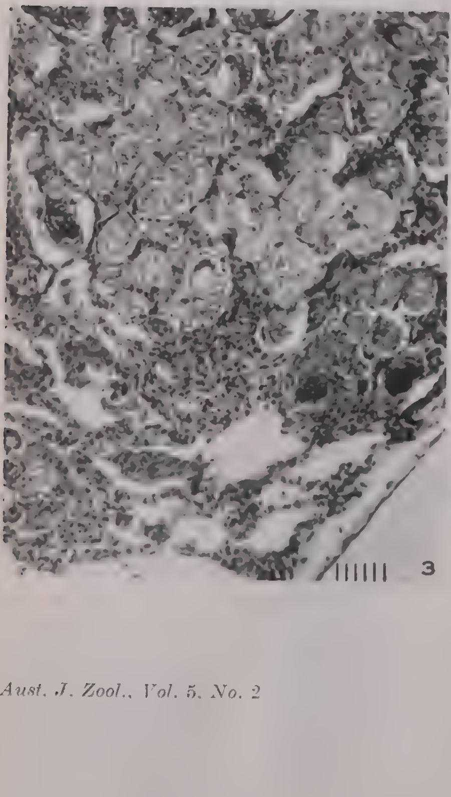

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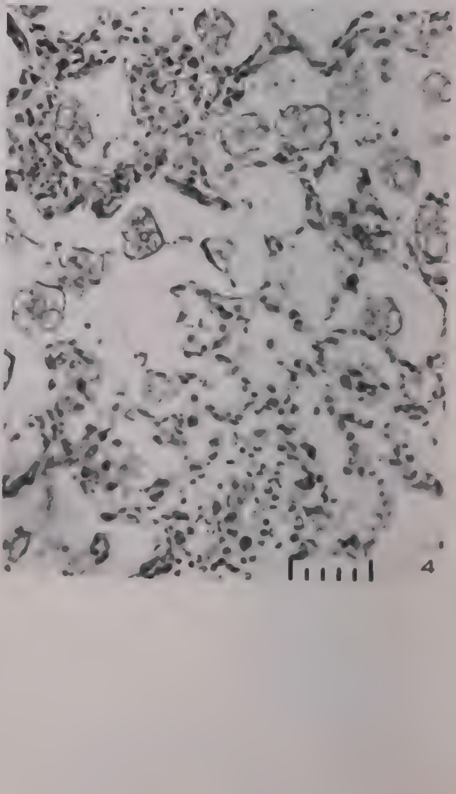

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Explanation of Plates 1 and 2

Plate 1

Figures 3–6 are to the same scale. Each small division on the scale in Figures 1 and 5 represents 10 μ. *a*, Anus; *e.d.*, externo-dorsal ray; *gub.*, gubernaculum; *int.*, intestine; *l*, lateral rays; *sp.*, spicules; *ut.*, uterus; *v*, ventral rays; *vu.*, vulva

Fig. 1.—Section of lung of kitten killed on 32nd day after infection, showing portions of adult worms.

Fig. 2.—Portion of gastro-splenic ligament of mouse, 13 days after ingesting infected slugs, showing third-stage larvae encysted at edge of a deposit of fat. Photographed unfixed in saline.

Fig. 3.—*A. abstrusus* adult female, anterior end.

Fig. 4.—*A. abstrusus* adult female, posterior end.

Fig. 5.—*A. abstrusus* adult male, posterior end, lateral view.

Fig. 6.—*A. abstrusus* adult male, posterior end, ventral view.

Plate 2

Each small division on the scale in Figures 3 and 4 represents 10 μ

Fig. 1.—Transverse section of right inferior lobe of lung of kitten 4 months after infection. *n*, *n′*, Parasitic nodules. × 3.

Fig. 2.—Parasitic nodule *n* in Figure 1 enlarged.

Fig. 3.—Portion of same nodule under higher magnification.

Fig. 4.—Section of lung of another kitten showing scattered ova and cell accumulations in alveoli.

THE ECOLOGY OF *ONCOPERA FASCICULATA* (WALKER) (LEPIDOPTERA: HEPIALIDAE) IN SOUTH AUSTRALIA

IV. FLUCTUATIONS IN NUMBERS DURING 1948–1955 AND AN ANALYSIS OF THEIR CAUSES

By P. E. Madge*

[*Manuscript received September* 28, 1956]

Summary

The development of the pastoral industry in County Grey has increased the numbers of *Oncopera fasciculata* (Walker) in the area. Even during unfavourable periods between outbreaks the numbers are greater than they used to be. Although the outbreak of 1948–50 was the greatest that has been recorded, not all of the suitable places were occupied during the outbreak, nor was all of the food eaten except in restricted areas. With food and shelter in excess of needs, the numbers reached during 1948–50 depended mainly on weather.

Predators have very little influence on the numbers of *O. fasciculata* except when unfavourable wet weather forces larvae, prepupae, and pupae from their subterranean burrows. No parasites were found during this study.

The evidence suggests that an increase in the numbers of *O. fasciculata* in any one generation may be expected when there are fewer than about 45 days without rain during the late egg and early larval stages (October–December). Extensive damage to pastures may be expected when there is a series of such years; this has happened only once during the past 95 years (1947–49), and was followed by the outbreak of 1948–50.

The numbers of *O. fasciculata* on the poorly drained soils are likely to increase whenever a dry, late winter (fewer than about 2 or 3 days of waterlogging during July–September) is followed by a wet, late spring.

Changes in the distribution and abundance of *O. fasciculata* during 1948–55 are described and a probable explanation is put forward.

I. Introduction

The distribution and abundance of *Oncopera fasciculata* (Walker) in the lower south-east of South Australia were estimated by making yearly surveys and counting the numbers of larvae in sample plots taken in representative areas. This information is summarized in Section II. A striking feature of the results was the changes in the distribution of the denser populations. This is illustrated by the annual maps and the notes accompanying them.

In the second part of this paper these data have been reviewed in relation to the experimental results that have been reported in earlier papers (Madge 1956*a*, 1956*b*, 1956*c*), and a general explanation of the distribution and abundance of *O. fasciculata* has been given. For this analysis the environment has been divided into four components: shelter, food, other animals, and weather.

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II. Description of the Distribution and Numbers of O. fasciculata during 1948–55

No thorough surveys were made during 1948–49 of the distribution of *O. fasciculata* in the south-east of South Australia but information obtained from other members of the Entomology Department and departmental records of the Waite Institute, farmers in the district, and reports in the Mt. Gambier newspaper enabled reasonably accurate maps to be drawn of the distribution and numbers of the species for these two years. It is likely that *O. fasciculata* was more abundant than is indicated on the maps (Figs. 1 and 2) for these years.

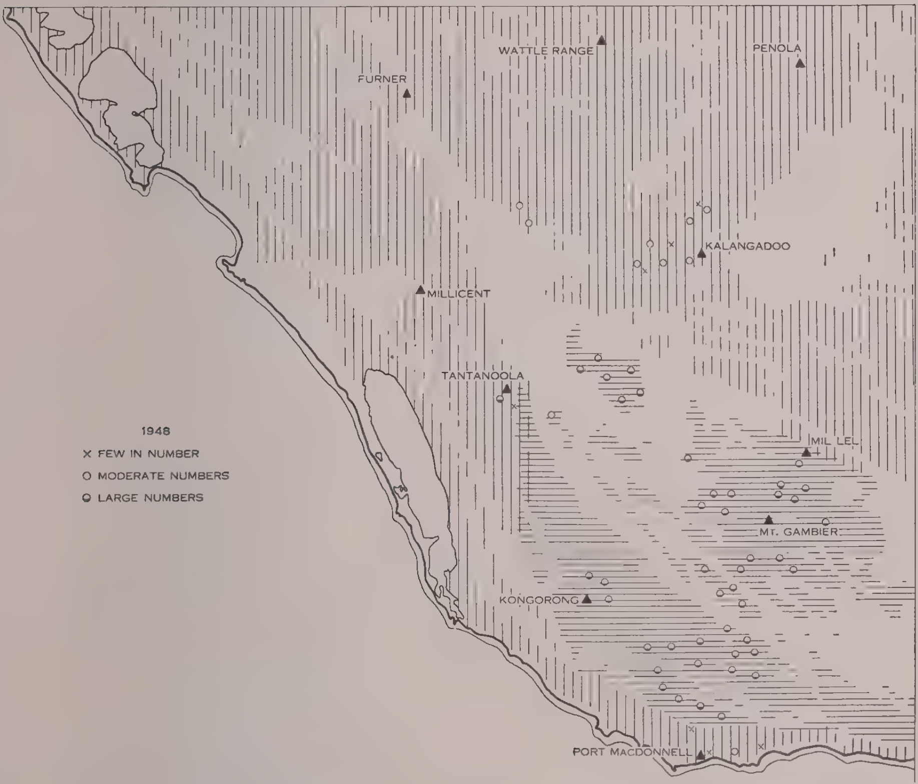

Fig. 1.—Distribution of *O. fasciculata* in 1948. Poorly drained soils are shown by vertical hatching and well-drained soils by horizontal hatching. Clear areas are places where *O. fasciculata* is not likely to survive, such as sclerophyll forests, pine plantations, sand dunes, etc. The species was in plague numbers on the well-drained soils.

During 1950–55 frequent trips were made to observe the insect in the field and to record the places where it was living and its abundance. The main surveys were done in July, when larvae were fully grown. The method used was to stop about every 2 miles along the road and inspect the adjoining land, when the numbers of larvae in several square-foot samples were recorded. They were classified as being either scarce, few, moderate, or large. Damage to herbage could not be used as a criterion of the numbers of larvae in a field because the amount of growth was dependent on weather. In some years when the weather favours a good growth of herbage, the plants may be able to "keep ahead" of the larvae with the result that little damage is apparent, even though there are many larvae feeding on the plants. During a year with weather unfavourable for pasture plants, the same numbers of larvae in the same paddock are likely to eat most of the herbage and cause severe damage.

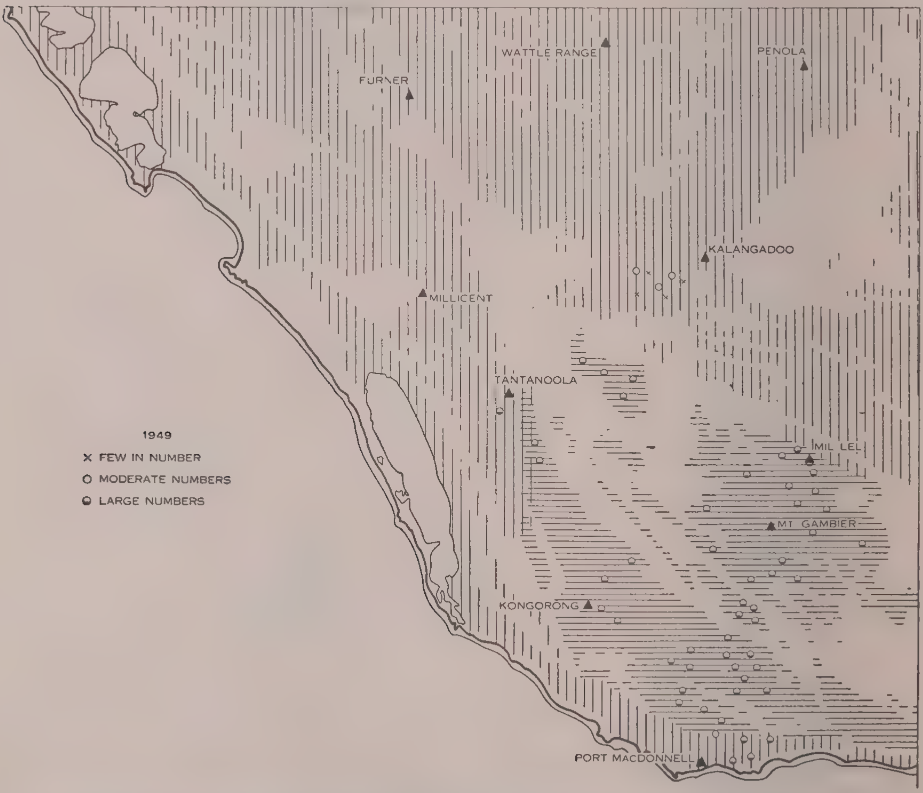

Fig. 2.—Distribution of *O. fasciculata* in 1949. Damage was restricted mainly to improved pastures on the well-drained soils.

A survey in May 1950, as well as in other years, showed that there were no larvae in such places as pine plantations, coastal sand dunes, dense forest and scrub, or other places where there was no herbage. As no larvae were found in these situations, and as it is very unlikely that they would be found there, the surveys were restricted mainly to natural grasslands and developed pastures.

Maps of County Grey were drawn showing the stranded dunes, forest reserves, lakes, and other places extremely unfavourable for the insect, and this portion of the county is shown unhatched on the maps (Figs. 1–8). The rest of the county is mainly under natural grasslands or developed pastures, and on the maps this land has been divided into poorly drained and well-drained soils (see Madge 1956*a*). Poorly drained soils are indicated by vertical hatching, and well-drained soils by horizontal hatching. The insect is not distributed over all of this land because portions of the poorly drained soils are much too wet for it to become established, nor is it found on cropped land. Some parts of the county could not be visited because there were no roads, and it was assumed that in these undeveloped areas *O. fasciculata* was rare or absent.

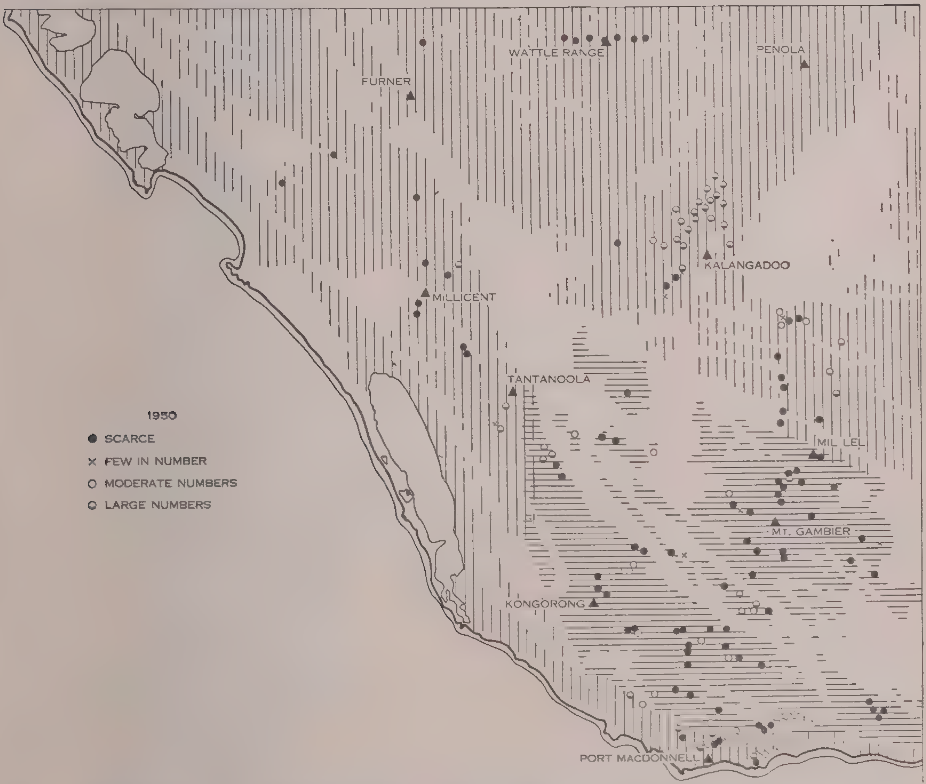

Fig. 3.—Distribution of *O. fasciculata* in July 1950. Larvae were numerous on the poorly drained soils and caused severe damage to pastures near Kalangadoo; there were fewer larvae on the well-drained soils. Note that the distribution is almost the opposite to that shown for 1948 and 1949.

In 1948 there were very large numbers of *O. fasciculata* on the well-drained soils around Mt. Gambier, and thousands of acres of improved pastures were severely damaged (Fig. 1). Some of the worst damage was near Mt. Schank (between Mt. Gambier and Port MacDonnell) and Port MacDonnell (Plate 1, Fig. 1), but pastures north of Mt. Gambier and at Kongorong also were severely attacked. The insect was less numerous on the poorly drained soils and appeared to be restricted mainly to the higher, drier land.

Larvae again caused severe damage to improved pastures in the Mt. Gambier–Mt. Schank area in 1949 (Fig. 2). There were large numbers in a small area south of Tantanoola and at Eight Mile Creek, east of Port MacDonnell (Plate 1, Fig. 2), and moderate numbers were found at Kalangadoo, which lies in typical poorly drained country. In 1948 and 1949 larvae were most numerous in places where there were improved pastures; some of the largest numbers were found where land was being developed for closer settlement by returned servicemen (see Section III(*a*)).

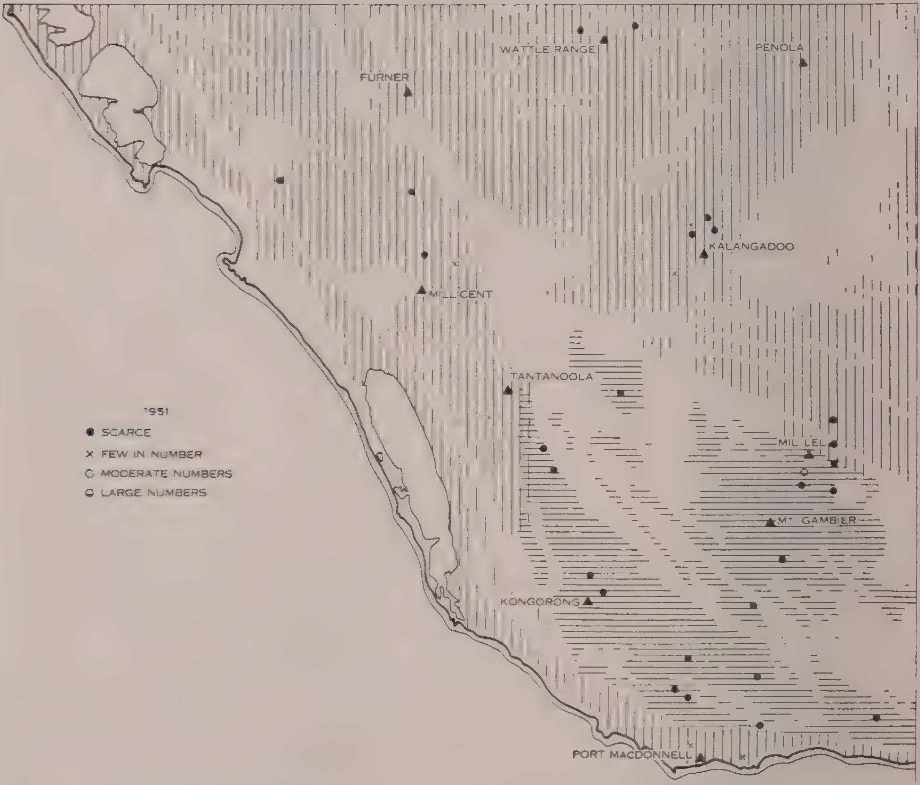

Fig. 4.—Distribution of *O. fasciculata* in July 1951. Larvae were very scarce and only one place was found where they were in numbers large enough to cause economic loss.

The survey in July 1950 showed that *O. fasciculata* was most abundant on the poorly drained soils (Fig. 3). This was the first time that large numbers were found in the district around Kalangadoo, and also the first time that the insect was found at Wattle Range. Much of the damage to herbage at Kalangadoo was restricted to the lower portions of land, but larvae were also easy to find on the higher banks of land (*c*. two to three larvae per 1 sq. ft sample). Larvae were also found in large numbers on the poorly drained soil at the soldier settlement of Pleasant Park, north of Mil Lel. Directly east of this area, in Victoria, and in much of the rest of the Western District of that State, herbage on many thousands of acres of land was severely damaged. There were fewer larvae on the well-drained soils north-east of Mt. Gambier and around Mt. Schank than in 1949, and the largest numbers were in the lower situations. Only a few places were found where larvae were numerous enough (*c.* 10–12 per sample) to cause economic loss.

Surveys made early in 1951 indicated that there had been a great reduction in the numbers of *O. fasciculata*. The main survey in July (Fig. 4) showed that there was only one situation, near Mil Lel, where severe damage was done to herbage. The numbers of larvae here averaged about 10 per sample. Larvae were scarce in the southern portion of the county and were restricted mainly to the lower, wetter situations on the well-drained soils. Larvae were also less numerous on the poorly drained soils around Kalangadoo, but in a few small situations there were enough to cause damage. Although large numbers of adults had been seen mating on stubble on a property near Mil Lel the previous September, there were few larvae in July. The mean of 15 samples taken here was slightly more than one larva per sample.

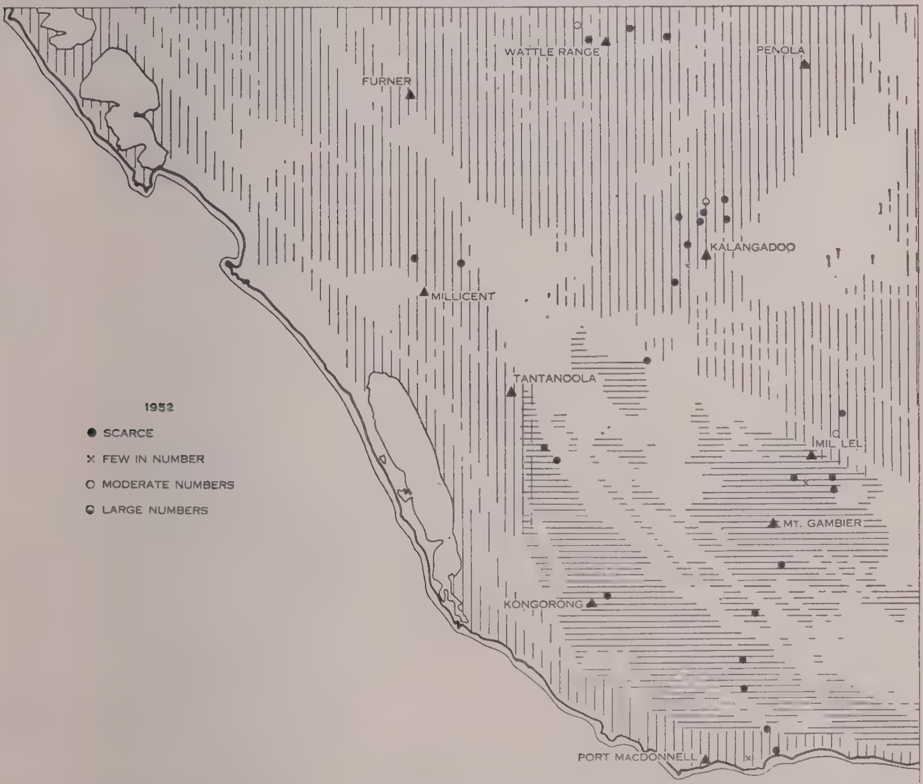

Fig. 5.—Distribution of *O. fasciculata* in August 1952. The species was slightly more abundant on the poorly drained soils.

In 1952 *O. fasciculata* was scarce in most parts of the county but a few local situations were found where there were moderate numbers of larvae (Fig. 5). Large numbers were found near Kalangadoo on a paddock that had been understocked the previous year. In the rest of the Kalangadoo district larvae were scarce or few in numbers. The largest numbers of larvae in 1952 were found in the wetter situations on both the well-drained and poorly drained soils.

The survey in July 1953 showed that larvae of *O. fasciculata* were more abundant than they had been since 1950 (Fig. 6). Minor damage was done to herbage on the well-drained soils at Mil Lel and Mt. Schank, and also on the low-lying soils at Kalangadoo, Wattle Range, and Millicent. Although there were moderate numbers of larvae in other situations, their feeding was masked by the abundant growth of herbage. Numbers around Kalangadoo were about the same as in 1952, but the distribution was restricted mainly to the higher land.

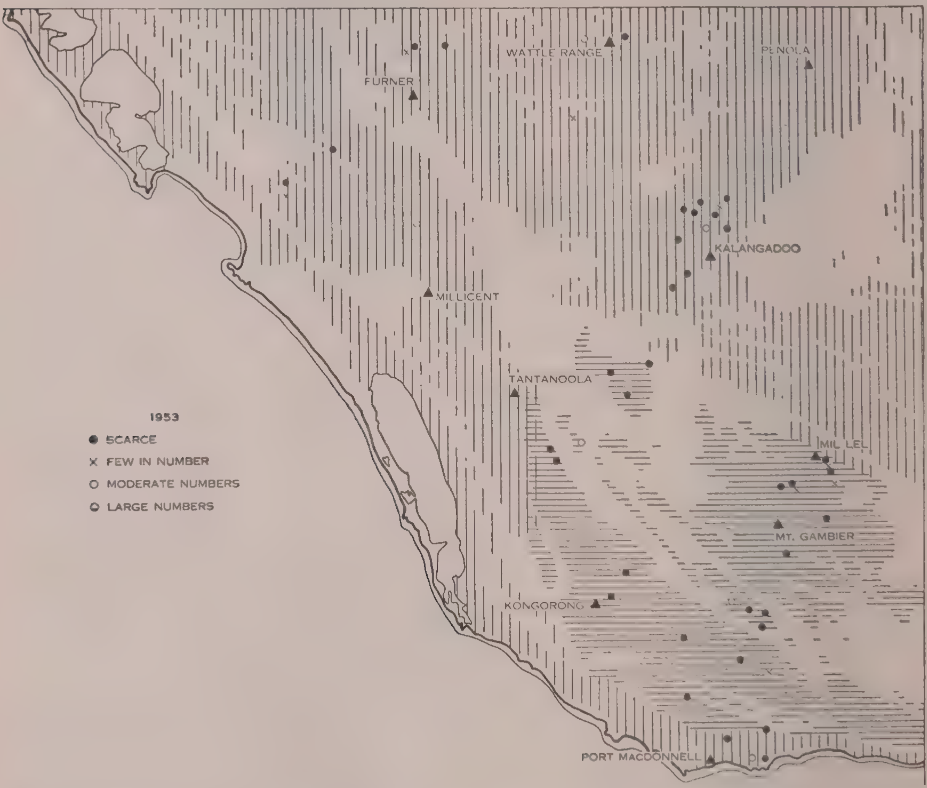

Fig. 6.—Distribution of *O. fasciculata* in July 1953. There had been a higher survival rate on both the poorly drained and well-drained soils.

In July 1954 several places were found where there were large numbers of *O. fasciculata*, both on well-drained and poorly drained soils (Fig. 7). Sampling in the area between Mt. Gambier and Port MacDonnell showed that there was a wider distribution of larvae than in the previous generation, and there were larger numbers in some of the lower situations. Numbers on the poorly drained soils around Kalangadoo were much the same as in the previous generation, but larvae were found mainly on the higher land; one paddock was heavily infested. There were large numbers of larvae on a property midway between Kalangadoo and Furner, where about 150 acres of herbage had been severely damaged. The soil here is a low-lying, poorly drained sand with banks of higher land; damage to herbage was restricted to this higher land.

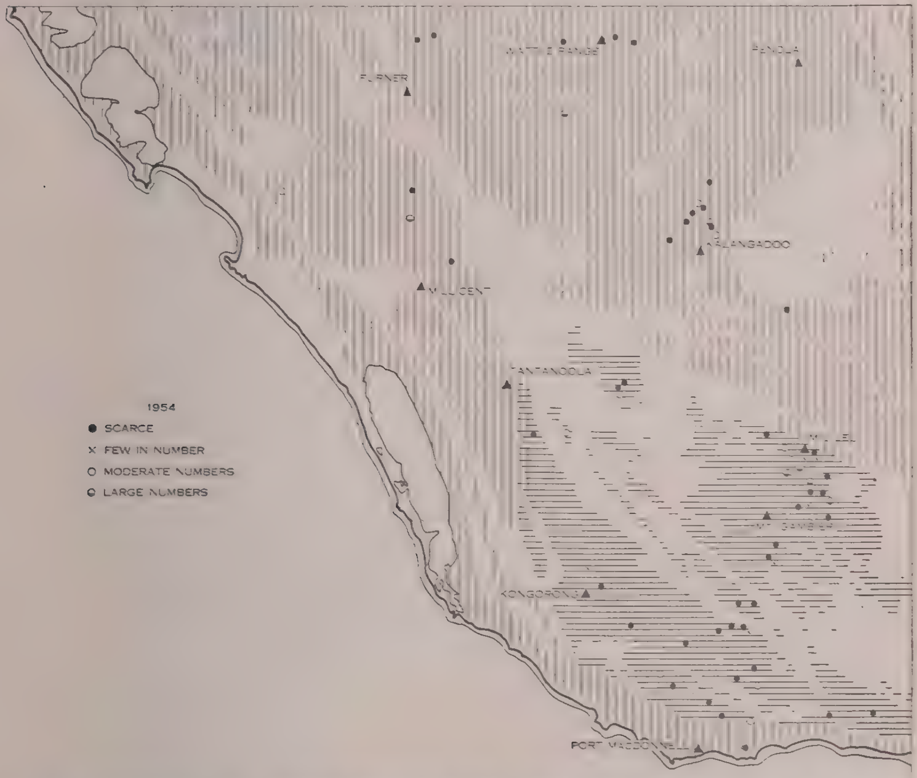

Fig. 7.—Distribution of *O. fasciculata* in July 1954. Numbers were maintained on both types of soil; large numbers were found on improved pastures near Kalangadoo and at Mil Lel.

Results of the survey in July 1955 are shown in Figure 8. The largest numbers of *O. fasciculata* were found in low-lying situations on both the well-drained and poorly drained soils. In other places the distribution of the insect was much the same as in the previous generation, but larvae were less numerous.

III. Analysis of the Environment of O. fasciculata: Its Influence on the Distribution and Numbers of the Species

(a) *Shelter*

The sort of place where a larva of *O. fasciculata* is living influences its chance to survive the hazards associated with excessive dryness or excessive wetness. For example, some situations may contain suitable breeding sites and abundant food for larvae but the herbage may not provide sufficient protection against unfavourable weather, and few individuals survive. It would appear that this interaction of local situation with weather helps to explain why the species is found only in small numbers on natural grasslands even during years when the weather is favourable, and why it is able to increase so greatly in numbers on improved pastures during favourable years. Native grasses have a tussocky habit and in natural grasslands there is a large proportion of bare ground (Plate 1, Fig. 3). In this sort of place there is not likely to be sufficient protection against dryness and heat. Where pastures have been improved by the introduction of clovers and grasses the herbage is tall and dense during the spring, unless it has been heavily grazed by sheep or cut for hay.

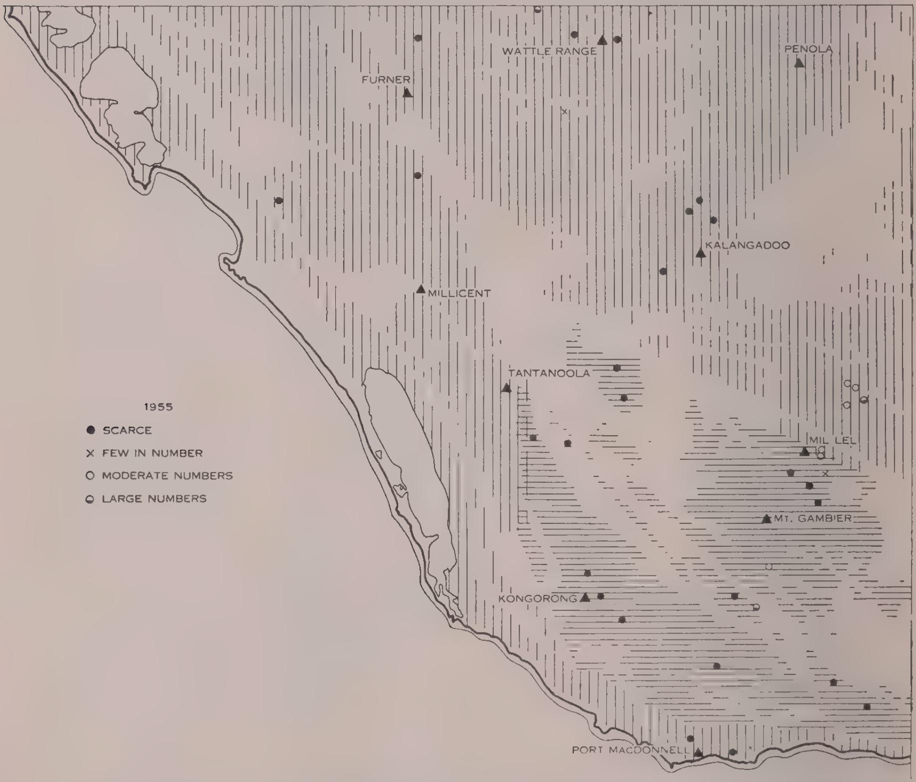

Fig. 8.—Distribution of *O. fasciculata* in July 1955. The insect was less abundant than in 1954 and the largest numbers were found in the low-lying areas of both the well-drained and poorly drained soils.

Many instances of the influence of surface cover on the distribution and numbers of *O. fasciculata* were seen during this study. Herbage on a paddock at Wattle Range was not grazed during the spring of 1952 so that it could be cut and baled for hay. The herbage was not cut, and larvae of *O. fasciculata* were numerous enough to cause moderate damage in this paddock the following winter. A property near Kalangadoo had contained large numbers of *O. fasciculata* in 1952. The suggestion was made to the owner that he try to have as little herbage as possible on his land during the spring, which was done. One large paddock was cut for hay and the cuttings removed by November. Livestock were concentrated on the remaining paddocks and had kept the herbage grazed rather low in comparison with nearby paddocks on other properties, where heavy grazing was not done. There were very few larvae on this property in 1953, and it appeared that in this instance the proper management of the property, in relation to the requirements of *O. fasciculata*, helped to prevent what might have been a serious loss of feed during the winter. A paddock near Kalangadoo was closed for hay during the spring of 1953 and was cut in December. The cuttings were not removed, however, and larvae were found here in large numbers in 1954. On a nearby property one paddock was cut for hay in November and the hay baled and removed soon afterwards. All of the remaining paddocks but one were closely grazed during the spring. In 1954 there were few larvae on the mown and heavily grazed paddocks but large numbers were found on the remaining paddock that had carried only a few sheep during the spring.

An unusual combination of circumstances, which were associated with the development of land for soldier settlement and which are not likely to occur again, made large areas of country especially favourable to *O. fasciculata* during 1947–49. (The following information was obtained from Mr. F. Pearson, District Agricultural Adviser for the lower south-east of South Australia.) Most of the land for closer settlement was obtained from large properties south of Mt. Schank, south-east of Tantanoola, north-east of Mt. Gambier, and around Kalangadoo. Pastures on this land were poor in quality because before World War II it was not a common practice to use superphosphate except in particular paddocks, e.g. lambing paddocks, and this fertilizer was very scarce during the war. It is very likely that most of the land taken over for soldier settlement received its first liberal application of superphosphate in 1947. This treatment, together with the sowing of grasses and clovers, resulted in about a 50 per cent. increase in the bulk growth of pasture plants, for the fertilizer promoted the growth of subterranean clover (*Trifolium subterraneum* L.), and a large amount of seed was produced. There were no soldier settlers or livestock on the blocks that year. The blocks received a second application of superphosphate in 1948, which resulted in about a 200 per cent. increase in growth of pasture plants; there was more clover and, because of the greater amount of nitrogen available, an increased growth of grasses. Some settlers moved on to their blocks late in 1948 but it was not until 1950 that sheep were introduced. The result of this development was that during 1947–49 there was a very great increase in the amount of herbage on soldier settlement blocks, and this cover, together with the favourable late springs of these years (see later), favoured the multiplication of *O. fasciculata*.

On the low-lying, poorly drained soils, the environment during late spring and early summer is not as dry as on the well-drained situations. However, another hazardous period for the species is during the late winter and early spring, when the poorly drained soils may become waterlogged. Even when there is extensive flooding some individuals are not exposed to the wetness because they are living on the higher, drier banks of land. The sort of pasture growth is also important because a larva may escape the wetness by crawling upwards into tussocks of grass.

Before County Grey was settled and developed there were few places providing shelter for the insect during the critical October–December period (see Section III (*d*)) that were not too wet in the winter and early spring. Even on the wetter portions of the well-drained land there probably was a high death rate in most years because the sparse growth of native grasses did not provide suitable protection against dryness. Numbers on the poorly drained soils might have been restricted mainly to places where there were tussocky grasses enabling larvae to escape when the area became waterlogged.

As native grasses were replaced by introduced clovers and grasses, more situations, especially on the well-drained soils, became favourable for *O. fasciculata* because this growth provided a better protection against dryness and heat. Also, the artificial drainage of the poorly drained soils resulted in less of this land being flooded, enabling the insect to survive over a wider area.

Nevertheless, there remain in County Grey extensive tracts of country that are not suitable for *O. fasciculata.* Tussocky grasses are sparsely distributed over natural sclerophyll forest and these probably are suitable food for the larvae. Although such country has been searched quite widely, only one larva of *O. fasciculata* was ever found in natural sclerophyll forest. Indeed, this sort of place must be very unfavourable for the establishment of *O. fasciculata* because suitable food is scarce, and there is little or no protection against unfavourable weather during the egg and early larval stages.

Similarly, larvae of *O. fasciculata* were not found in pine plantations. Bracken fern (*Pteridium aquilinum* (L.) Kuhn) is associated with young plantings, but as the trees grow the fern disappears and only a mat of pine needles is found under them. One would not expect to find *O. fasciculata* here because it is not the sort of place where mating flights or egg laying occur, and there is no food for the larvae.

Undeveloped, poorly drained soils are unfavourable places for the species because of the poor quality of the food and the wetness during the winter and spring. When these soils have been developed and are carrying introduced grasses and clovers, the environment still remains unfavourable because of the risk of drowning.

Even in those areas that are more favourable for *O. fasciculata*, because they are not subject to flooding and the practice of maintaining permanent pastures provides a consistent supply of good food and shelter, the species is still unable to increase in numbers except when the weather is especially favourable (see Section III(*d*)).

(*b*) *Food*

Larvae have been found so numerous that practically all of the green herbage was eaten and then they fed on the old, dead stubble remaining from the previous year, on bracken, or on certain weeds. In such cases it is likely that the poor quality of the food would cause a reduction in the fecundity of the adults that emerge (see Andrewartha and Birch 1954, ch. 11). It is possible that before the introduction of better quality clovers and grasses into County Grey, the numbers of *O. fasciculata* were restricted, among other things, by the poor quality of the native grasses, for these are very fibrous and are low in protein (Tiver and Crocker 1951).

Food usually is abundant, but in most places during most years *O. fasciculata* is rare compared with the food available. Because of the patchy distribution of the larvae it sometimes happens that local situations are eaten bare. When this happens, larvae apparently do not leave their burrows or runways but remain there until food again becomes available or they die. This has been seen many times—areas eaten bare, with larvae still in their burrows, although abundant food was no more than a few feet or yards away. It is quite certain that the larvae did not, even in these circumstances, crawl across to the nearby herbage, feed, and then return to their burrows, for there was no webbing or any other means enabling larvae to return to their burrows. Also, there was little evidence of activity around the mouths of the burrows, and the silken caps covering the mouths were collapsed.

(*c*) *Other Animals*

(i) *Members of the Same Species*.—In the field, *O. fasciculata* is distributed patchily, and even in places where it is scarce, larvae are more likely to be found in groups than as isolated individuals. This sort of distribution may be explained by the behaviour of the gravid female. After mating, usually on upright stems or leaves, she crawls down into the herbage and lays her eggs under surface debris. For this reason the largest numbers of larvae are found where pasture growth was tall during the flight season (September–October), and in these places the distribution of the population is determined largely by where the females came to rest.

Places that appear to be ideal for the development of *O. fasciculata* may not be colonized simply because gravid females are not attracted there. An example of this sort of place was seen in 1955. North-east of Mil Lel a portion of a paddock had been ploughed in 1953, left fallow, and ploughed again in 1954. It was sown with clover and grasses soon afterwards and the whole paddock was lightly grazed during 1954. During the flight season of *O. fasciculata* that year, the newly sown portion of the paddock carried a low, dense cover of clover and grass while the unploughed area carried a high, dense cover of Yorkshire fog (*Holcus lanatus* L.) and clover. During the spring and summer of 1955 the paddock was lightly stocked with sheep and cattle. Larvae of *O. fasciculata* were very abundant there in July 1955, but were restricted to the portion of the paddock that had carried the tall, dense cover of Yorkshire fog. The contrast between the sorts of cover can be seen in Plate 1, Figure 4. The area in the foreground was sown with clover and grasses, and the herbage is short and dense. In the background is the old pasture which is dominated by a dense cover of Yorkshire fog. Ten random square-foot samples were dug from each situation. The numbers of *O. fasciculata* in the high, dense growth ranged from two to nine per sq. ft, with a mean of 3·9, while no larva was found in 10 samples from the low, dense growth. No larva was found in this situation even after further intensive searching.

The behaviour of the adults in mating mainly on tall herbage probably had a high survival value before the development of improved pastures. It resulted in eggs being laid in tussocks of native grasses where the eggs and larvae were less exposed to dryness and larvae emerged with food nearby, and the tussocks provided a retreat for larvae if the soil became waterlogged. Also, this behaviour resulted in the moths congregating during mating flights, and so the females were more likely to be fertilized.

Today, with the widespread development of improved pastures, most larvae of *O. fasciculata* live in places that provide uniform protection and food (Madge 1956*c*). As solitary larvae are less likely to experience a shortage of food than members of a dense group, it is difficult to imagine any advantage from having eggs laid in groups. The disadvantage may be all the greater if, as seems likely, the fecundity has increased with the appearance of a better-quality food, and so more eggs might be expected to be laid now by a female than when there were only native grasses.

(ii) *Other Species of Animals*.—Among the other animals associated with pastures in County Grey, sheep, rabbits, and the pasture cockchafer (*Aphodius howitti* Hope) are potentially important components in the environment of *O. fasciculata*. Sheep usually are of minor importance because as herbage becomes scarce the sheep are removed, leaving abundant food for the insect. However, sheep feeding on herbage during the spring, when the eggs and young larvae are on the surface of the ground, might kill these stages directly by crushing them and indirectly by removing the herbage and exposing them to unfavourable weather. This latter danger is likely only when the sheep are in large enough numbers to keep the herbage closely grazed. With its subterranean habit, the older larva is in little danger of being crushed by sheep or larger animals.

The area around rabbit warrens usually is unfavourable for *O. fasciculata* because the rabbits keep the herbage closely grazed, and there is a large proportion of bare ground. But the total of these areas is not usually large. The less concentrated feeding by the rabbits which occurs further from their warrens has little influence on *O. fasciculata* except when the rabbits are unusually numerous or the pastures unusually poor. Low rainfall, especially during autumn, may reduce the growth of herbage and thus increase the importance of the rabbit in the environment of the insect.

Larvae of *A. howitti* shelter in subterranean burrows and emerge to feed on herbage. The behaviour of these larvae differs from that of *O. fasciculata* larvae in that they migrate from places where most of the herbage has been eaten and so produce bare patches which increase in size during the feeding period (about April–September). These two insects have been found living close together when the total numbers were few, but when one was abundant the other was scarce. Females of *A. howitti* seem to prefer open areas in a paddock for egg laying while most eggs of *O. fasciculata* are laid where herbage is tall and dense. The mating flights of *A. howitti* occur during February–March. At this time larvae of *O. fasciculata* are still living under a cover of stubble and herbage because they do not start to feed actively until after the autumn rains (Madge 1956*c*). Thus the differences in behaviour with respect to the places where they lay their eggs tends to keep the two species separate.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Figures 3–6 are to the same scale. Each small division on the scale in Figures 1 and 5 represents 10 μ. *a*, Anus; *e.d.*, externo-dorsal ray; *gub.*, gubernaculum; *int.*, intestine; *l*, lateral rays; *sp.*, spicules; *ut.*, uterus; *v*, ventral rays; *vu.*, vulva

Fig. 1.—Section of lung of kitten killed on 32nd day after infection, showing portions of adult worms.

Fig. 2.—Portion of gastro-splenic ligament of mouse, 13 days after ingesting infected slugs, showing third-stage larvae encysted at edge of a deposit of fat. Photographed unfixed in saline.

Fig. 3.—*A. abstrusus* adult female, anterior end.

Fig. 4.—*A. abstrusus* adult female, posterior end.

Fig. 5.—*A. abstrusus* adult male, posterior end, lateral view.

Fig. 6.—*A. abstrusus* adult male, posterior end, ventral view.

PLATE 2

Each small division on the scale in Figures 3 and 4 represents 10 μ

Fig. 1.—Transverse section of right inferior lobe of lung of kitten 4 months after infection. *n*, *n′*, Parasitic nodules. ×3.

Fig. 2.—Parasitic nodule *n* in Figure 1 enlarged.

Fig. 3.—Portion of same nodule under higher magnification.

Fig. 4.—Section of lung of another kitten showing scattered ova and cell accumulations in alveoli.

THE ECOLOGY OF *ONCOPERA FASCICULATA* (WALKER) (LEPIDOPTERA: HEPIALIDAE) IN SOUTH AUSTRALIA

IV. FLUCTUATIONS IN NUMBERS DURING 1948–1955 AND AN ANALYSIS OF THEIR CAUSES

By P. E. Madge*

[*Manuscript received September 28, 1956*]

Summary

The development of the pastoral industry in County Grey has increased the numbers of *Oncopera fasciculata* Walker, in the area. Even during unfavourable periods between outbreaks the numbers are greater than they used to be. Although the outbreak of 1948–50 was the greatest that has been recorded, not all of the suitable places were occupied during the outbreak, nor was all of the food eaten except in restricted areas. With food and shelter in excess of needs, the numbers reached during 1948–50 depended mainly on weather.

Predators have very little influence on the numbers of *O. fasciculata* except when unfavourable wet weather forces larvae, prepupae, and pupae from their subterranean burrows. No parasites were found during this study.

The evidence suggests that an increase in the numbers of *O. fasciculata* in any one generation may be expected when there are fewer than about 45 days without rain during the late egg and early larval stages (October–December). Extensive damage to pastures may be expected when there is a series of such years; this has happened only once during the past 95 years (1947–49), and was followed by the outbreak of 1948–50.

The numbers of *O. fasciculata* on the poorly drained soils are likely to increase whenever a dry, late winter (fewer than about 2 or 3 days of waterlogging during July–September) is followed by a wet, late spring.

Changes in the distribution and abundance of *O. fasciculata* during 1948–55 are described and a probable explanation is put forward.

I. INTRODUCTION

The distribution and abundance of *Oncopera fasciculata* (Walker) in the lower south-east of South Australia were estimated by making yearly surveys and counting the numbers of larvae in sample plots taken in representative areas. This information is summarized in Section II. A striking feature of the results was the changes in the distribution of the denser populations. This is illustrated by the annual maps and the notes accompanying them.

In the second part of this paper these data have been reviewed in relation to the experimental results that have been reported in earlier papers (Madge 1956*a*, 1956*b*, 1956*c*), and a general explanation of the distribution and abundance of *O. fasciculata* has been given. For this analysis the environment has been divided into four components: shelter, food, other animals, and weather.

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II. DESCRIPTION OF THE DISTRIBUTION AND NUMBERS OF O. FASCICULATA DURING 1948–55

No thorough surveys were made during 1948–49 of the distribution of *O. fasciculata* in the south-east of South Australia but information obtained from other members of the Entomology Department and departmental records of the Waite Institute, farmers in the district, and reports in the Mt. Gambier newspaper enabled reasonably accurate maps to be drawn of the distribution and numbers of the species for these two years. It is likely that *O. fasciculata* was more abundant than is indicated on the maps (Figs. 1 and 2) for these years.

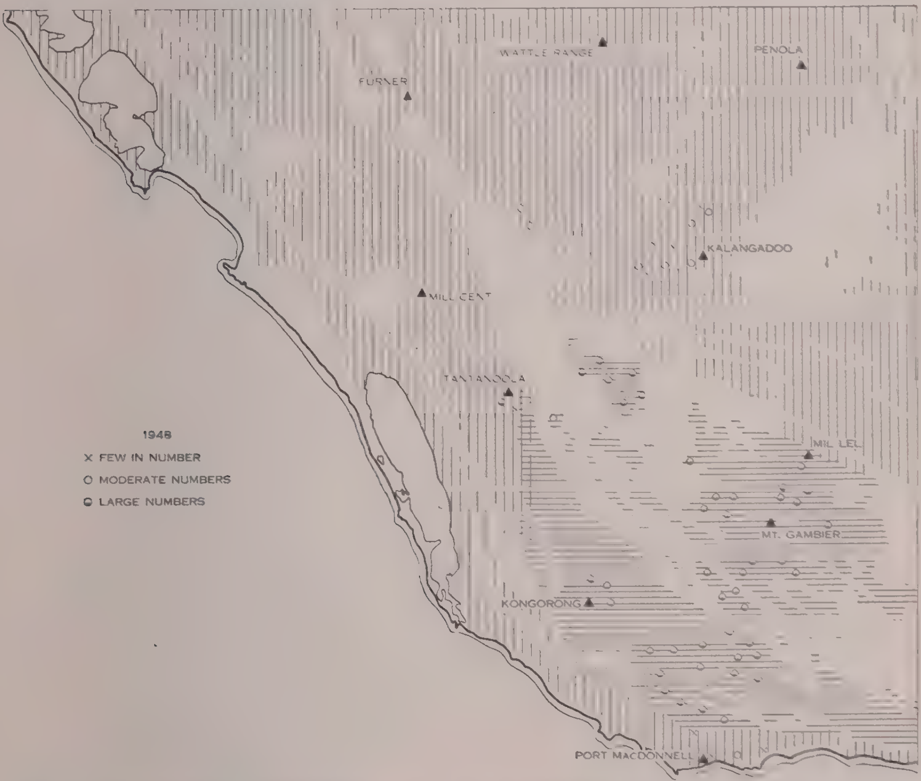

Fig. 1.—Distribution of *O. fasciculata* in 1948. Poorly drained soils are shown by vertical hatching and well-drained soils by horizontal hatching. Clear areas are places where *O. fasciculata* is not likely to survive, such as sclerophyll forests, pine plantations, sand dunes, etc. The species was in plague numbers on the well-drained soils.

During 1950–55 frequent trips were made to observe the insect in the field and to record the places where it was living and its abundance. The main surveys were done in July, when larvae were fully grown. The method used was to stop about every 2 miles along the road and inspect the adjoining land, when the numbers of larvae in several square-foot samples were recorded. They were classified as being either scarce, few, moderate, or large. Damage to herbage could not be used as a criterion of the numbers of larvae in a field because the amount of growth was dependent on weather. In some years when the weather favours a good growth of herbage, the plants may be able to "keep ahead" of the larvae with the result that little damage is apparent, even though there are many larvae feeding on the plants. During a year with weather unfavourable for pasture plants, the same numbers of larvae in the same paddock are likely to eat most of the herbage and cause severe damage.

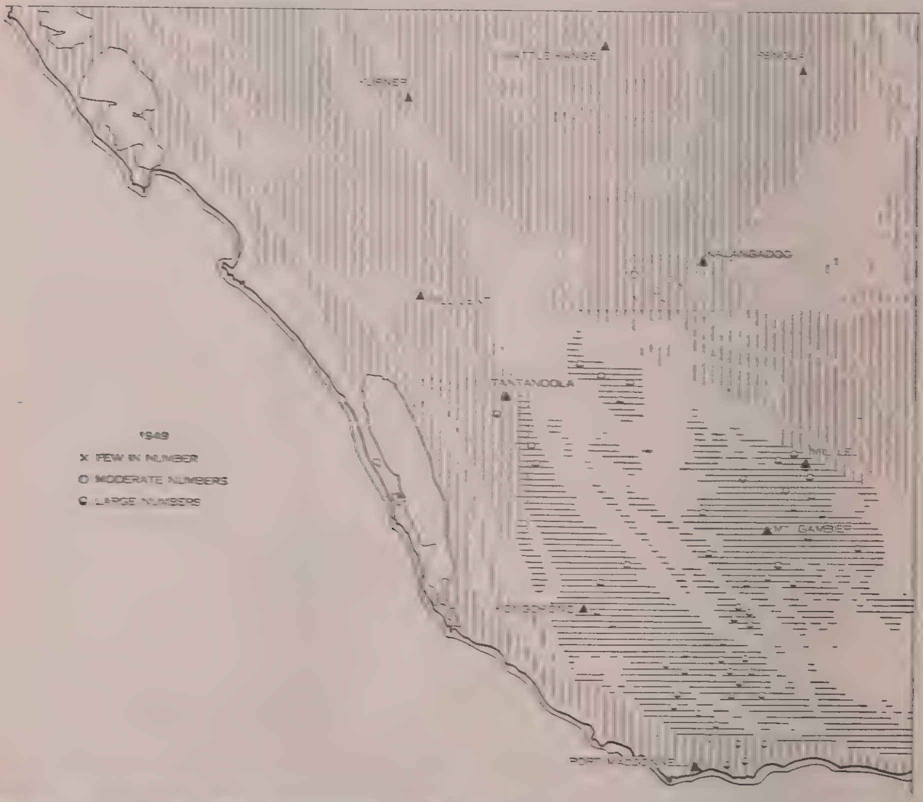

Fig. 2.—Distribution of *O. fasciculata* in 1949. Damage was restricted mainly to improved pastures on the well-drained soils.

A survey in May 1950, as well as in other years, showed that there were no larvae in such places as pine plantations, coastal sand dunes, dense forest and scrub, or other places where there was no herbage. As no larvae were found in these situations, and as it is very unlikely that they would be found there, the surveys were restricted mainly to natural grasslands and developed pastures.

Maps of County Grey were drawn showing the stranded dunes, forest reserves, lakes, and other places extremely unfavourable for the insect, and this portion of the county is shown unhatched on the maps (Figs. 1–8). The rest of the county is mainly under natural grasslands or developed pastures, and on the maps this land has been divided into poorly drained and well-drained soils (see Madge 1956c). Poorly drained soils are indicated by vertical hatching, and well-drained soils by horizontal hatching. The insect is not distributed over all of this land because portions of the poorly drained soils are much too wet for it to become established, nor is it found on cropped land. Some parts of the county could not be visited because there were no roads, and it was assumed that in these undeveloped areas *O. fasciculata* was rare or absent.

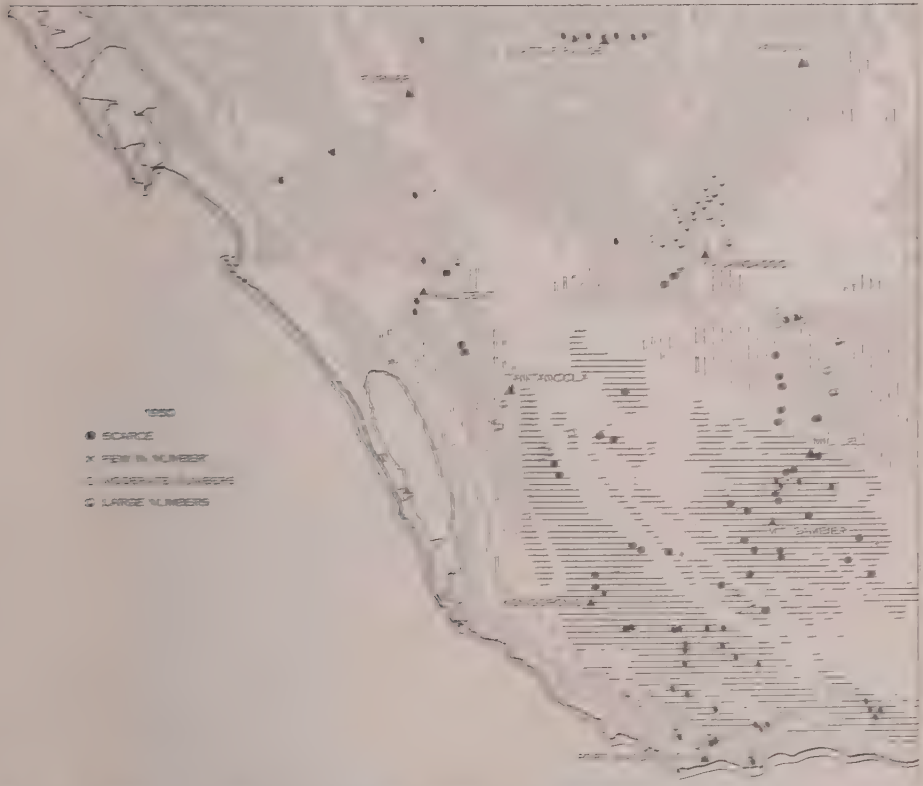

Fig. 3—Distribution of *O. fasciculata* in July 1950. Larvae were numerous on the poorly drained soils and caused severe damage to pastures near Kalangadoo; there were fewer larvae on the well-drained soils. Note that the distribution is almost the opposite of that shown for 1948 and 1949.

In 1948 there were very large numbers of *O. fasciculata* on the well-drained soils around Mt. Gambier, and thousands of acres of improved pastures were severely damaged (Fig. 1). Some of the worst damage was near Mt. Schank (between Mt. Gambier and Port MacDonnell) and Port MacDonnell (Plate 1, Fig. 1), but pastures north of Mt. Gambier and at Kongorong also were severely attacked. The insect was less numerous on the poorly drained soils and appeared to be restricted mainly to the higher, drier land.

Larvae again caused severe damage to improved pastures in the Mt. Gambier-Mt. Schank area in 1949 (Fig. 2). There were large numbers in a small area south of Tantanoola and at Eight Mile Creek, east of Port MacDonnell (Plate I, Fig. 2), and moderate numbers were found at Kalangadoo, which lies in typical poorly drained country. In 1948 and 1949 larvae were most numerous in places where there were improved pastures; some of the largest numbers were found where land was being developed for closer settlement by returned servicemen (see Section III(*a*)).

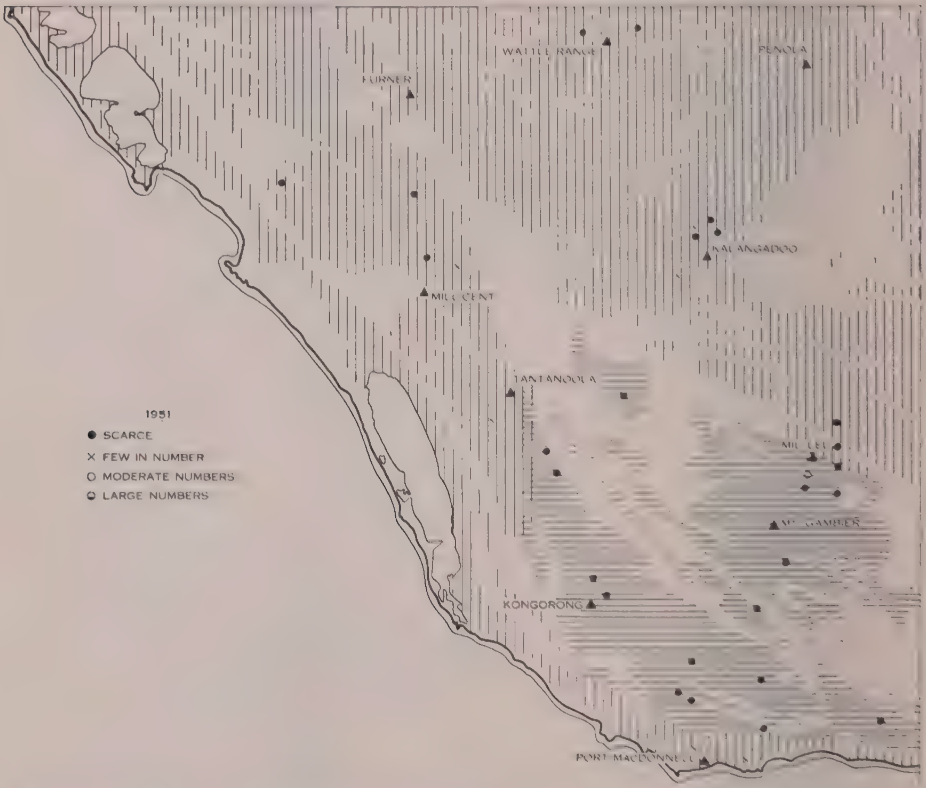

Fig. 4. Distribution of *O. fasciculata* in July 1951. Larvae were very scarce and only one place was found where they were in numbers large enough to cause economic loss.

The survey in July 1950 showed that *O. fasciculata* was most abundant on the poorly drained soils (Fig. 3). This was the first time that large numbers were found in the district around Kalangadoo, and also the first time that the insect was found at Wattle Range. Much of the damage to herbage at Kalangadoo was restricted to the lower portions of land, but larvae were also easy to find on the higher banks of land (*c*. two to three larvae per 1 sq. ft sample). Larvae were also found in large numbers on the poorly drained soil at the soldier settlement of Pleasant Park, north of Mil Lel. Directly east of this area, in Victoria, and in much of the rest of the Western District of that State, herbage on many thousands of acres of land was severely damaged. There were fewer larvae on the well-drained soils north-east of Mt. Gambier and around Mt. Schank than in 1949, and the largest numbers were in the lower situations. Only a few places were found where larvae were numerous enough (*c.* 10–12 per sample) to cause economic loss.

Surveys made early in 1951 indicated that there had been a great reduction in the numbers of *O. fasciculata*. The main survey in July (Fig. 4) showed that there was only one situation, near Mil Lel, where severe damage was done to herbage. The numbers of larvae here averaged about 10 per sample. Larvae were scarce in the southern portion of the county and were restricted mainly to the lower, wetter situations on the well-drained soils. Larvae were also less numerous on the poorly drained soils around Kalangadoo, but in a few small situations there were enough to cause damage. Although large numbers of adults had been seen mating on stubble on a property near Mil Lel the previous September, there were few larvae in July. The mean of 15 samples taken here was slightly more than one larva per sample.

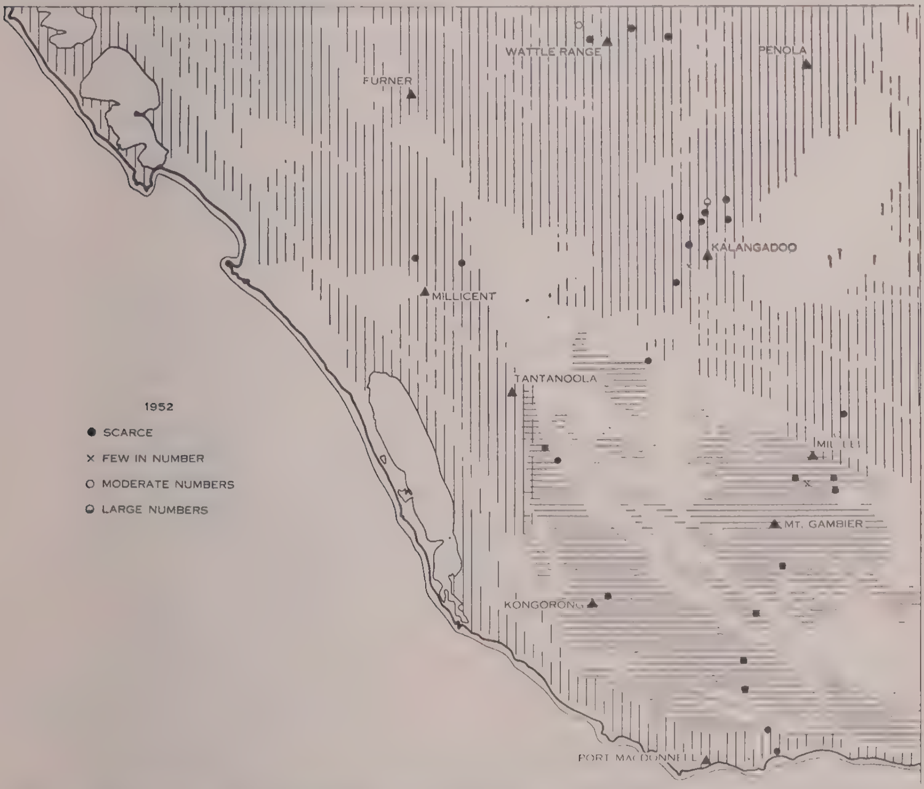

Fig. 5.—Distribution of *O. fasciculata* in August 1952. The species was slightly more abundant on the poorly drained soils.

In 1952 *O. fasciculata* was scarce in most parts of the county but a few local situations were found where there were moderate numbers of larvae (Fig. 5). Large numbers were found near Kalangadoo on a paddock that had been understocked the previous year. In the rest of the Kalangadoo district larvae were scarce or few in numbers. The largest numbers of larvae in 1952 were found in the wetter situations on both the well-drained and poorly drained soils.

The survey in July 1953 showed that larvae of *O. fasciculata* were more abundant than they had been since 1950 (Fig. 6). Minor damage was done to herbage on the well-drained soils at Mil Lel and Mt. Schank, and also on the low-lying soils at Kalangadoo, Wattle Range, and Millicent. Although there were moderate numbers of larvae in other situations, their feeding was masked by the abundant growth of herbage. Numbers around Kalangadoo were about the same as in 1952, but the distribution was restricted mainly to the higher land.

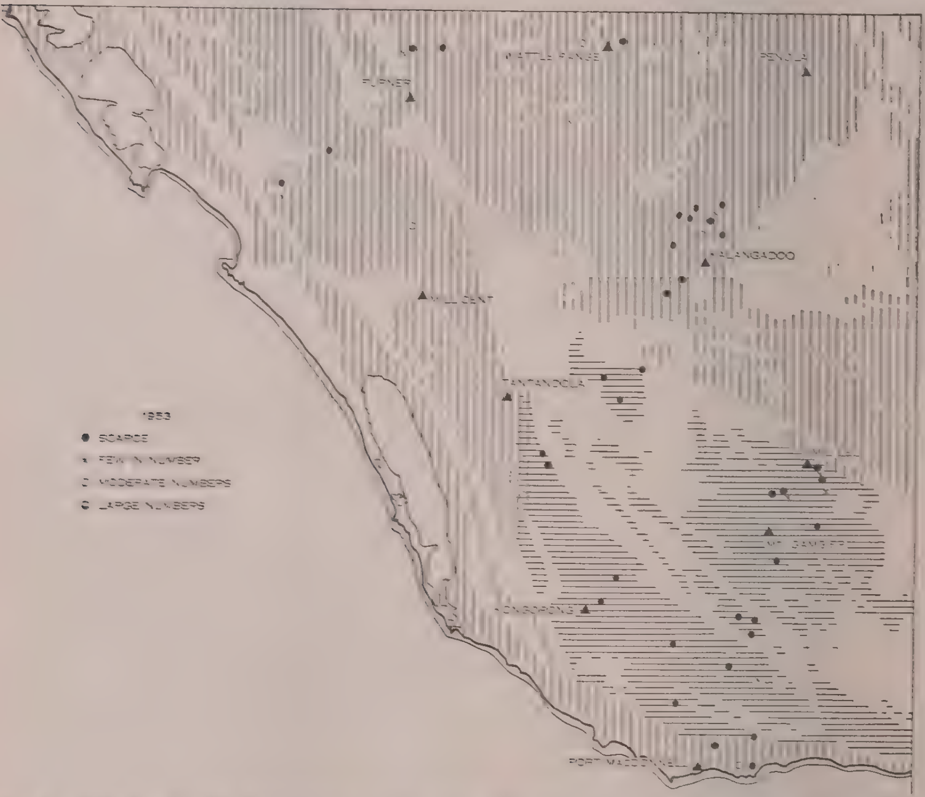

Fig. 6.—Distribution of *O. fasciculata* in July 1953. There had been a higher survival rate on both the poorly drained and well-drained soils.

In July 1954 several places were found where there were large numbers of *O. fasciculata*, both on well-drained and poorly drained soils (Fig. 7). Sampling in the area between Mt. Gambier and Port MacDonnell showed that there was a wider distribution of larvae than in the previous generation, and there were larger numbers in some of the lower situations. Numbers on the poorly drained soils around Kalangadoo were much the same as in the previous generation, but larvae were found mainly on the higher land: one paddock was heavily infested. There were large numbers of larvae on a property midway between Kalangadoo and Furner, where about 150 acres of herbage had been severely damaged. The soil here is a low-lying, poorly drained sand with banks of higher land; damage to herbage was restricted to this higher land.

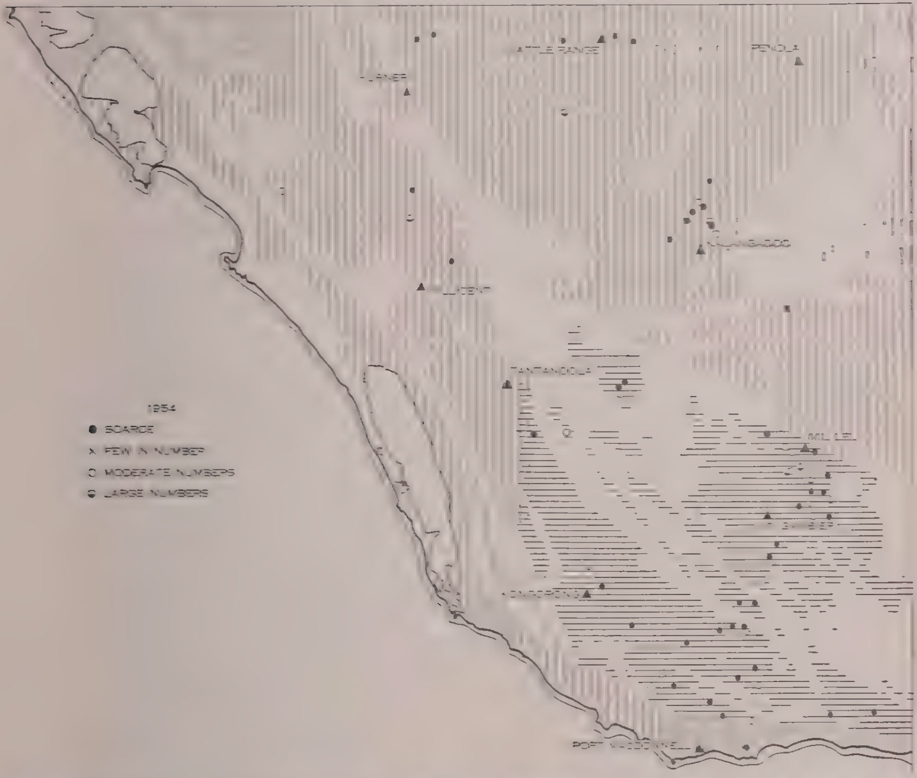

Fig. 7.—Distribution of *O. fasciculata* in July 1954. Numbers were maintained on both types of soil: large numbers were found on improved pastures near Kalangadoo and at Mil Lel.

Results of the survey in July 1955 are shown in Figure 8. The largest numbers of *O. fasciculata* were found in low-lying situations on both the well-drained and poorly drained soils. In other places the distribution of the insect was much the same as in the previous generation, but larvae were less numerous.

III. Analysis of the Environment of O. fasciculata: Its Influence on the Distribution and Numbers of the Species

(a) *Shelter*

The sort of place where a larva of *O. fasciculata* is living influences its chance to survive the hazards associated with excessive dryness or excessive wetness. For example, some situations may contain suitable breeding sites and abundant food for larvae but the herbage may not provide sufficient protection against unfavourable weather, and few individuals survive. It would appear that this interaction of local situation with weather helps to explain why the species is found only in small numbers on natural grasslands even during years when the weather is favourable, and why it is able to increase so greatly in numbers on improved pastures during favourable years. Native grasses have a tussocky habit and in natural grasslands there is a large proportion of bare ground (Plate 1, Fig. 3). In this sort of place there is not likely to be sufficient protection against dryness and heat. Where pastures have been improved by the introduction of clovers and grasses the herbage is tall and dense during the spring, unless it has been heavily grazed by sheep or cut for hay.

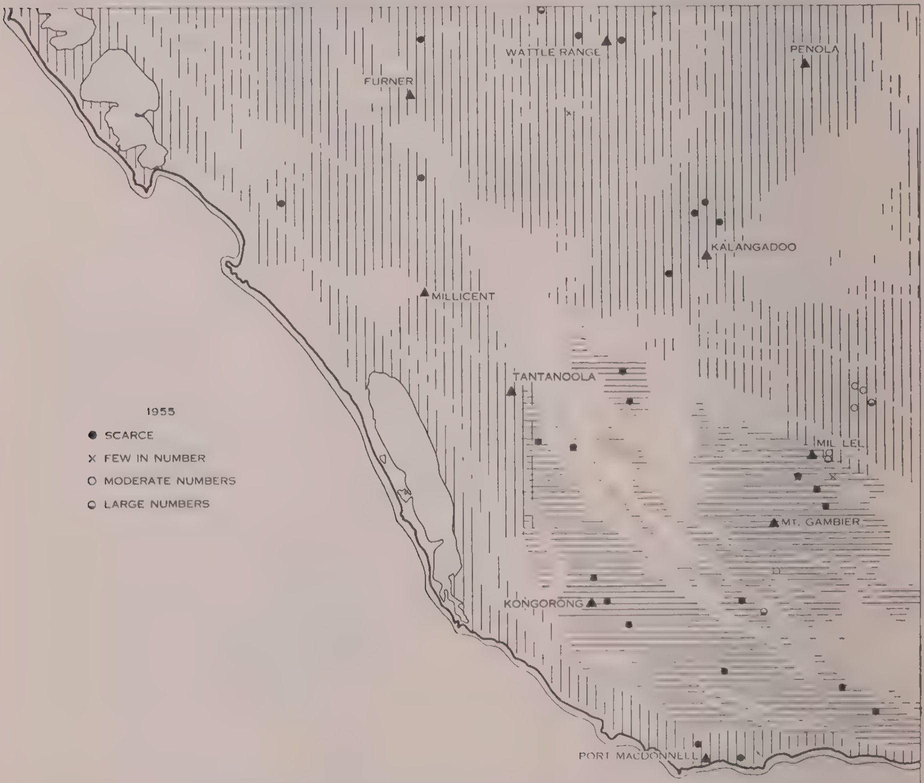

Fig. 8.—Distribution of *O. fasciculata* in July 1955. The insect was less abundant than in 1954 and the largest numbers were found in the low-lying areas of both the well-drained and poorly drained soils.

Many instances of the influence of surface cover on the distribution and numbers of *O. fasciculata* were seen during this study. Herbage on a paddock at Wattle Range was not grazed during the spring of 1952 so that it could be cut and baled for hay. The herbage was not cut, and larvae of *O. fasciculata* were numerous enough to cause moderate damage in this paddock the following winter. A property near Kalangadoo had contained large numbers of *O. fasciculata* in 1952. The suggestion was made to the owner that he try to have as little herbage as possible on his land during the spring, which was done. One large paddock was cut for hay and the cuttings removed by November. Livestock were concentrated on the remaining paddocks and had kept the herbage grazed rather low in comparison with nearby paddocks on other properties, where heavy grazing was not done. There were very few larvae on this property in 1953, and it appeared that in this instance the proper management of the property, in relation to the requirements of *O. fasciculata*, helped to prevent what might have been a serious loss of feed during the winter. A paddock near Kalangadoo was closed for hay during the spring of 1953 and was cut in December. The cuttings were not removed, however, and larvae were found here in large numbers in 1954. On a nearby property one paddock was cut for hay in November and the hay baled and removed soon afterwards. All of the remaining paddocks but one were closely grazed during the spring. In 1954 there were few larvae on the mown and heavily grazed paddocks but large numbers were found on the remaining paddock that had carried only a few sheep during the spring.

An unusual combination of circumstances, which were associated with the development of land for soldier settlement and which are not likely to occur again, made large areas of country especially favourable to *O. fasciculata* during 1947–49. (The following information was obtained from Mr. F. Pearson, District Agricultural Adviser for the lower south-east of South Australia.) Most of the land for closer settlement was obtained from large properties south of Mt. Schank, south-east of Tantanoola, north-east of Mt. Gambier, and around Kalangadoo. Pastures on this land were poor in quality because before World War II it was not a common practice to use superphosphate except in particular paddocks, e.g. lambing paddocks, and this fertilizer was very scarce during the war. It is very likely that most of the land taken over for soldier settlement received its first liberal application of superphosphate in 1947. This treatment, together with the sowing of grasses and clovers, resulted in about a 50 per cent. increase in the bulk growth of pasture plants, for the fertilizer promoted the growth of subterranean clover (*Trifolium subterraneum* L.), and a large amount of seed was produced. There were no soldier settlers or livestock on the blocks that year. The blocks received a second application of superphosphate in 1948, which resulted in about a 200 per cent. increase in growth of pasture plants; there was more clover and, because of the greater amount of nitrogen available, an increased growth of grasses. Some settlers moved on to their blocks late in 1948 but it was not until 1950 that sheep were introduced. The result of this development was that during 1947–49 there was a very great increase in the amount of herbage on soldier settlement blocks, and this cover, together with the favourable late springs of these years (see later), favoured the multiplication of *O. fasciculata*.

On the low-lying, poorly drained soils, the environment during late spring and early summer is not as dry as on the well-drained situations. However, another hazardous period for the species is during the late winter and early spring, when the poorly drained soils may become waterlogged. Even when there is extensive flooding some individuals are not exposed to the wetness because they are living on the higher, drier banks of land. The sort of pasture growth is also important because a larva may escape the wetness by crawling upwards into tussocks of grass.

Before County Grey was settled and developed there were few places providing shelter for the insect during the critical October–December period (see Section III (*d*)) that were not too wet in the winter and early spring. Even on the wetter portions of the well-drained land there probably was a high death rate in most years because the sparse growth of native grasses did not provide suitable protection against dryness. Numbers on the poorly drained soils might have been restricted mainly to places where there were tussocky grasses enabling larvae to escape when the area became waterlogged.

As native grasses were replaced by introduced clovers and grasses, more situations, especially on the well-drained soils, became favourable for *O. fasciculata* because this growth provided a better protection against dryness and heat. Also, the artificial drainage of the poorly drained soils resulted in less of this land being flooded, enabling the insect to survive over a wider area.

Nevertheless, there remain in County Grey extensive tracts of country that are not suitable for *O. fasciculata*. Tussocky grasses are sparsely distributed over natural sclerophyll forest and these probably are suitable food for the larvae. Although such country has been searched quite widely, only one larva of *O. fasciculata* was ever found in natural sclerophyll forest. Indeed, this sort of place must be very unfavourable for the establishment of *O. fasciculata* because suitable food is scarce, and there is little or no protection against unfavourable weather during the egg and early larval stages.

Similarly, larvae of *O. fasciculata* were not found in pine plantations. Bracken fern (*Pteridium aquilinum* (L.) Kuhn) is associated with young plantings, but as the trees grow the fern disappears and only a mat of pine needles is found under them. One would not expect to find *O. fasciculata* here because it is not the sort of place where mating flights or egg laying occur, and there is no food for the larvae.

Undeveloped, poorly drained soils are unfavourable places for the species because of the poor quality of the food and the wetness during the winter and spring. When these soils have been developed and are carrying introduced grasses and clovers, the environment still remains unfavourable because of the risk of drowning.

Even in those areas that are more favourable for *O. fasciculata*, because they are not subject to flooding and the practice of maintaining permanent pastures provides a consistent supply of good food and shelter, the species is still unable to increase in numbers except when the weather is especially favourable (see Section III(*d*)).

(*b*) *Food*

Larvae have been found so numerous that practically all of the green herbage was eaten and then they fed on the old, dead stubble remaining from the previous year, on bracken, or on certain weeds. In such cases it is likely that the poor quality of the food would cause a reduction in the fecundity of the adults that emerge (see Andrewartha and Birch 1954, ch. 11). It is possible that before the introduction of better quality clovers and grasses into County Grey, the numbers of *O. fasciculata* were restricted, among other things, by the poor quality of the native grasses, for these are very fibrous and are low in protein (Tiver and Crocker 1951).

Food usually is abundant, but in most places during most years *O. fasciculata* is rare compared with the food available. Because of the patchy distribution of the larvae it sometimes happens that local situations are eaten bare. When this happens, larvae apparently do not leave their burrows or runways but remain there until food again becomes available or they die. This has been seen many times—areas eaten bare, with larvae still in their burrows, although abundant food was no more than a few feet or yards away. It is quite certain that the larvae did not, even in these circumstances, crawl across to the nearby herbage, feed, and then return to their burrows, for there was no webbing or any other means enabling larvae to return to their burrows. Also, there was little evidence of activity around the mouths of the burrows, and the silken caps covering the mouths were collapsed.

(c) *Other Animals*

(i) *Members of the Same Species.*—In the field, *O. fasciculata* is distributed patchily, and even in places where it is scarce, larvae are more likely to be found in groups than as isolated individuals. This sort of distribution may be explained by the behaviour of the gravid female. After mating, usually on upright stems or leaves, she crawls down into the herbage and lays her eggs under surface debris. For this reason the largest numbers of larvae are found where pasture growth was tall during the flight season (September–October), and in these places the distribution of the population is determined largely by where the females came to rest.

Places that appear to be ideal for the development of *O. fasciculata* may not be colonized simply because gravid females are not attracted there. An example of this sort of place was seen in 1955. North-east of Mil Lel a portion of a paddock had been ploughed in 1953, left fallow, and ploughed again in 1954. It was sown with clover and grasses soon afterwards and the whole paddock was lightly grazed during 1954. During the flight season of *O. fasciculata* that year, the newly sown portion of the paddock carried a low, dense cover of clover and grass while the unploughed area carried a high, dense cover of Yorkshire fog (*Holcus lanatus* L.) and clover. During the spring and summer of 1955 the paddock was lightly stocked with sheep and cattle. Larvae of *O. fasciculata* were very abundant there in July 1955, but were restricted to the portion of the paddock that had carried the tall, dense cover of Yorkshire fog. The contrast between the sorts of cover can be seen in Plate 1, Figure 4. The area in the foreground was sown with clover and grasses, and the herbage is short and dense. In the background is the old pasture which is dominated by a dense cover of Yorkshire fog. Ten random square-foot samples were dug from each situation. The numbers of *O. fasciculata* in the high, dense growth ranged from two to nine per sq. ft, with a mean of 3·9, while no larva was found in 10 samples from the low, dense growth. No larva was found in this situation even after further intensive searching.

The behaviour of the adults in mating mainly on tall herbage probably had a high survival value before the development of improved pastures. It resulted in eggs being laid in tussocks of native grasses where the eggs and larvae were less exposed to dryness and larvae emerged with food nearby, and the tussocks provided a retreat for larvae if the soil became waterlogged. Also, this behaviour resulted in the moths congregating during mating flights, and so the females were more likely to be fertilized.

Today, with the widespread development of improved pastures, most larvae of *O. fasciculata* live in places that provide uniform protection and food (Madge 1956*c*). As solitary larvae are less likely to experience a shortage of food than members of a dense group, it is difficult to imagine any advantage from having eggs laid in groups. The disadvantage may be all the greater if, as seems likely, the fecundity has increased with the appearance of a better-quality food, and so more eggs might be expected to be laid now by a female than when there were only native grasses.

(ii) *Other Species of Animals.*—Among the other animals associated with pastures in County Grey, sheep, rabbits, and the pasture cockchafer (*Aphodius howitti* Hope) are potentially important components in the environment of *O. fasciculata*. Sheep usually are of minor importance because as herbage becomes scarce the sheep are removed, leaving abundant food for the insect. However, sheep feeding on herbage during the spring, when the eggs and young larvae are on the surface of the ground, might kill these stages directly by crushing them and indirectly by removing the herbage and exposing them to unfavourable weather. This latter danger is likely only when the sheep are in large enough numbers to keep the herbage closely grazed. With its subterranean habit, the older larva is in little danger of being crushed by sheep or larger animals.

The area around rabbit warrens usually is unfavourable for *O. fasciculata* because the rabbits keep the herbage closely grazed, and there is a large proportion of bare ground. But the total of these areas is not usually large. The less concentrated feeding by the rabbits which occurs further from their warrens has little influence on *O. fasciculata* except when the rabbits are unusually numerous or the pastures unusually poor. Low rainfall, especially during autumn, may reduce the growth of herbage and thus increase the importance of the rabbit in the environment of the insect.

Larvae of *A. howitti* shelter in subterranean burrows and emerge to feed on herbage. The behaviour of these larvae differs from that of *O. fasciculata* larvae in that they migrate from places where most of the herbage has been eaten and so produce bare patches which increase in size during the feeding period (about April–September). These two insects have been found living close together when the total numbers were few, but when one was abundant the other was scarce. Females of *A. howitti* seem to prefer open areas in a paddock for egg laying while most eggs of *O. fasciculata* are laid where herbage is tall and dense. The mating flights of *A. howitti* occur during February–March. At this time larvae of *O. fasciculata* are still living under a cover of stubble and herbage because they do not start to feed actively until after the autumn rains (Madge 1956*c*). Thus the differences in behaviour with respect to the places where they lay their eggs tends to keep the two species separate.

Larvae of other beetles of the family Scarabaeidae are pests of pastures in County Grey but seldom are found in the same places as larvae of *O. fasciculata*. The reason for this is not known, although it may be because of different preferences for oviposition sites.

Predators and parasites are considered to have very little influence on the numbers and distribution of *O. fasciculata*. No parasites were found and the most important predators (birds) were effective only when excessive wetness had forced larvae, prepupae, and pupae from their subterranean burrows (Madge 1956*a*).

(*d*) *Weather*

Under natural conditions eggs and young larvae of *O. fasciculata* may be exposed to excessive dryness, and mature larvae, prepupae, and pupae to excessive wetness. As was mentioned earlier, the intensity of the unfavourable weather is modified by the sort of plant growth on the surface of the ground (see also Geiger 1950, ch. 28). Few of the young stages of *O. fasciculata* survived in the field experiments described by Madge (1956*b*, 1956*c*) when they were under herbage that had been mown or thinned (the mowing treatment provided cover similar to that found on a mown, improved pasture, and the thinning treatment, cover on an unimproved pasture). These two treatments did not provide sufficient cover for the insect and the result was a high death rate caused by exposure to unfavourable weather. This result helps to explain why *O. fasciculata* seldom is found in large numbers in natural pastures or paddocks that have been heavily grazed or mown during the early spring. In these field experiments there was a high death rate (*c*. 90 per cent.) even where the eggs and larvae were under dense herbage. This high death rate probably was caused by dry weather during the late spring of 1951; that is, the weather of that year was so unfavourable that most individuals died even when they were in the most favourable situations.

Because these stages in the life cycle are so vulnerable to drought, the weather was analysed from records taken at Mt. Gambier during the period 1930–55 to find whether there was any relationship between changes in the numbers of *O. fasciculata* and changes in weather. Laboratory experiments showed that as the embryo develops it becomes more susceptible to loss of water, and that young larvae are more susceptible to loss of water than are the eggs. Therefore, weather data for October–December were analysed; this is the time of year when older eggs and young larvae are found in the field. Figure 9 shows for each year the number of days without rain during October–December.

This histogram shows the close agreement between the relative numbers of *O. fasciculata* and weather during the late spring; a higher survival rate is likely when there were few "dry" days. It appears that the numbers reported in 1935 resulted from very favourable weather during the previous spring. Reports indicated that *O. fasciculata* was relatively abundant in 1937, 1938, and 1940; Figure 9 shows that there were relatively few dry days during October–December in 1936 and 1939. Similarly, the great outbreak of *O. fasciculata* which lasted from 1948 to 1950 followed a sequence of years from 1944 to 1949 characterized by above-average rainfall and an unusually large number of "wet" days during the late springs of 1944 and

1947–49. The evidence suggests that an increase in the numbers of *O. fasciculata* in any one generation may be expected when there are fewer than about 45 days without rain during the previous October–December; a series of such years is likely to result in a great increase in the numbers of the species.

As the summer advances, larvae extend their burrows more deeply into the soil, thus gaining some protection from the dryness and heat of summer. Field experiments done in the autumn of 1954 showed that larvae remained relatively inactive in their burrows during periods of drought but became active and fed during wet periods (Madge 1956*c*). The greatest and most prolonged period of feeding occurred after the end of the summer drought. The amount of feeding was not measured directly at any other season of the year but the increase in length of larvae was measured in 1951. They increased in length by 26 per cent. during January, 45 per cent. during February–March, 28 per cent. during April, 55 per cent. during May, and 51 per cent. during June. These figures indicate an abrupt change in the relative growth rate from about 0·25 per month to about 0·5 per month with the break in the season, which occurred early in May. It seems that exposure to dryness not only kills many larvae but also retards the development of those that survive.

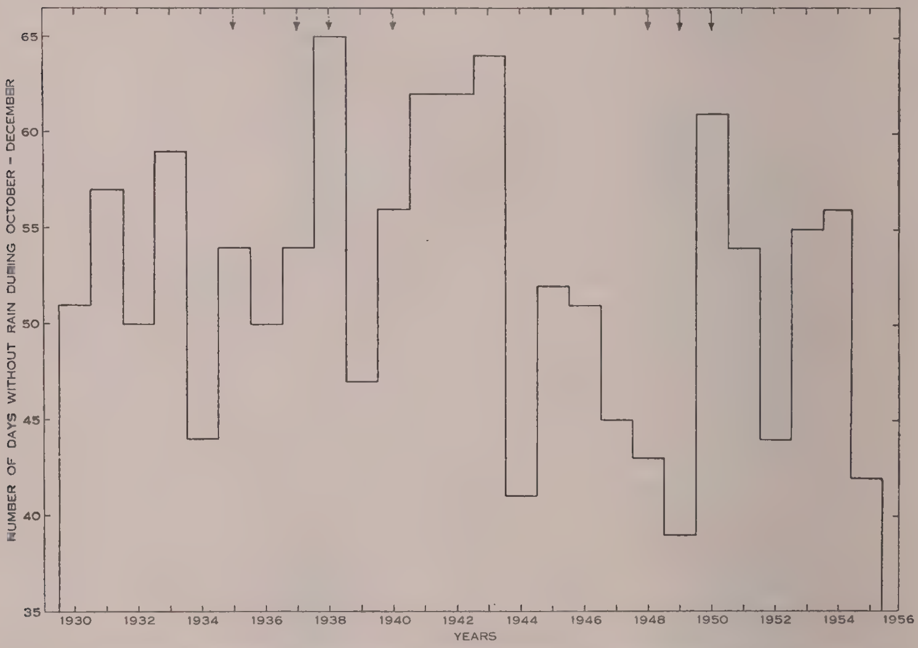

Fig. 9.—Histogram showing fluctuations in the numbers of unfavourable "dry" days at Mt. Gambier during the late egg–early larval stages (October–December). The relative abundance of *O. fasciculata* is shown above the histogram: arrows with solid shafts indicate large numbers, arrows with broken shafts indicate moderate numbers, and where there are no arrows the insect was scarce. For further explanation see text.

Other field studies done in the autumn of 1954 (Madge 1956*c*) showed that air in the lower portions of burrows of *O. fasciculata* larvae was nearly saturated with water vapour, and larvae sheltering there were in little danger of losing water. However, during a hot, dry day they might lose water when in the upper portions of their burrows or in one of the surface runways. These field studies were done in April, before the first substantial autumn rains. Weather in April is more favourable for the species than the summer months because it is cooler and wetter (see Table 1). Also, larvae are living closer to the surface of the ground during the summer and so are more exposed to dryness and heat. The building of subterranean burrows and covered runways may be an adaptation enabling *O. fasciculata* to survive in an environment which otherwise would be too dry. The same sort of adaptation is exhibited by termites and some ants.

TABLE 1

METEOROLOGICAL DATA FOR SUMMER MONTHS AND APRIL FOR MT. GAMBIER

Month	Mean Monthly Rainfall (in.)	Mean Monthly Evaporation (in.)	Mean Monthly Temperature (°F)
December	1·62	5·6	62·0
January	1·34	6·5	64·8
February	1·03	5·2	65·8
April	2·38	2·5	58·1

The advantage of living in a subterranean burrow is that a larva of *O. fasciculata* is protected from the dryness and heat prevailing above ground during summer. The disadvantage is that the larva may be drowned if the soil becomes waterlogged during winter. Waterlogging was not seen on the well-drained soils of County Grey but is of great importance in limiting the numbers of the insect on the poorly drained soils that may be flooded for part of the year. There is not much rain during the summer and it usually is not until late winter or early spring that the water-table may rise to the surface of the ground. In some situations the land may be under water for several weeks or longer. Even when the surface water has drained away, the soil remains very wet and a larva is not able to remain underground for long because there is water in its burrow. An unfavourably wet environment may cause a high death rate among larvae, prepupae, and pupae indirectly by forcing individuals from their burrows and exposing them to weather and predators and weakening them and making them more subject to attacks of virus, bacterial, and fungal diseases; or directly by drowning.

Many examples of waterlogging on poorly drained soils, and the subsequent drowning of larvae of *O. fasciculata*, were seen during 1950–53. Using daily rainfall data from Kalangadoo (typical of the poorly drained soils), values were obtained for the distribution and amount of rain needed to cause the waterlogging that was seen. This was done by considering both the total amount of rain that had fallen (with a subsequent rise in the water-table) and the large falls of rain that resulted in the surface flooding. Thus it was possible, by examining daily rainfall data for other years, to estimate the number of days when surface flooding probably occurred.

Fluctuations in the number of "wet" days during July–September at Kalangadoo for the years 1941–54 are shown in Figure 10. The almost complete absence of waterlogging during 1948–49, in addition to favourable weather during the late springs of these years (see Fig. 9), allowed a higher survival rate and probably was the cause of the outbreak of *O. fasciculata* on the poorly drained soils in 1950. It seems that, in this sort of country, *O. fasciculata* is likely to increase in numbers whenever a favourable late winter (no more than about 2 or 3 days of flooding during July–September) is followed by a favourable (i.e. moist) late spring.

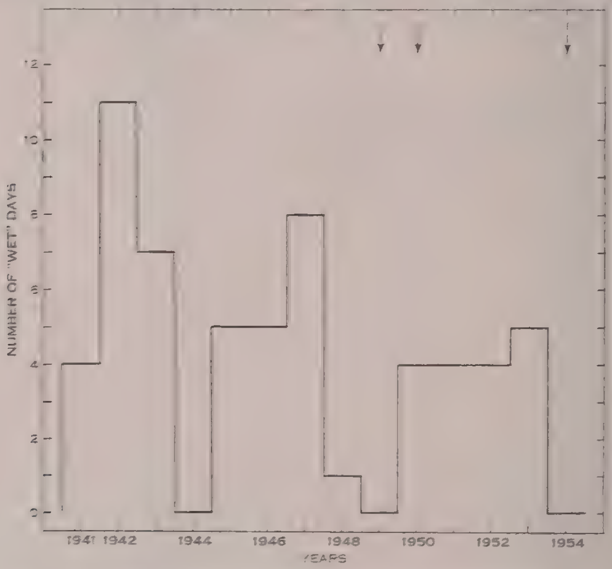

Fig. 10.—Histogram showing the numbers of unfavourable "wet" days at Kalangadoo during the mature larval, prepupal, and pupal stages (July–September). A day was considered as "wet" if flooding was likely to occur. Arrows as in Figure 9. For further explanation see text.

The probable explanation of changes in the distribution and abundance of *O. fasciculata* during 1948–55 is therefore as follows: During 1945–47 there probably were small to moderate numbers of the species on the well-drained soils but few on the poorly drained soils. The late spring of 1944 was very favourable but weather was less kind during this same period in 1945 and 1946. The late winter of 1944 was dry but there were about 5 days of flooding during the late winters of 1945 and 1946.

Weather during the late spring of 1947 was very favourable, resulting in a high survival rate of *O. fasciculata*, especially in the extensive area being developed for closer settlement. There were about 8 days of flooding during the previous winter, so in 1948 the insect was abundant on the well-drained soils but was restricted to the higher, drier portions of the poorly drained land.

The late winter and late spring of 1948 were very favourable, so in 1949 there was a great increase in the numbers of the species on both the well-drained and poorly drained soils. Late winter and late spring were again very favourable in 1949, resulting in a further increase in numbers on both sorts of soil in 1950. However, in 1950 most of the land under soldier settlement was carrying sheep and cattle. Feeding by these livestock resulted in a less favourable environment for *O. fasciculata* on the well-drained soils and probably is the explanation for the smaller numbers of the species on these soils in 1950.

Weather during the late winter of 1950 was unfavourable and that during the late spring was very unfavourable. This change in weather caused a great reduction in the numbers of the species on both sorts of soil in 1951. The late winter of 1951 was wet but weather was slightly kinder during the late spring; the highest survival rate in 1952 was on the higher portions of the poorly drained land.

The late winter of 1952 was unfavourable but the late spring was very favourable, and so in 1953 there was a high survival rate on the well-drained soils as well as on the higher portions of the poorly drained soils. The late spring of 1953 was not very favourable and in 1954 the numbers of *O. fasciculata* were maintained on the well-drained soils as well as on portions of the poorly drained soils that were not waterlogged during the wet late winter. The favourable late winter but unfavourable late spring of 1954 resulted in a decrease in numbers of the species on both sorts of soil in 1955.

IV. Conclusions

Although *O. fasciculata* has become generally more abundant during the past 30 years because man has provided a more favourable environment, the relative numbers and the distribution of the species are determined mainly by weather. The distribution is limited to the southernmost portion of the State where rainfall is reliable and comparatively abundant. Northwards from this region *O. fasciculata* becomes increasingly rare until the area is reached where individuals are never found, even though food is available. This distribution is closely associated with an environment which becomes drier during October–December, and thus less favourable for the species, as one travels northwards from County Grey.

In the more favourable southern region, *O. fasciculata* is restricted to situations where there are native grasses or introduced herbage plants. The largest numbers of the species are found where pastures are composed of introduced clovers and grasses because these plants provide the greatest amount of shelter against unfavourable weather in the late spring. Besides providing protection against unfavourable weather, the sort of pasture growth largely determines where mating takes place and where the gravid female lays her eggs, for there is a marked preference for places where the growth is upstanding. There may be a high survival rate of *O. fasciculata* in these favourable situations during a year with weather favourable for the species. A year would be favourable if the population were not exposed to extreme dryness during late spring (October–December) or to waterlogging during late winter (July–September).

An analysis of rainfall data from Mt. Gambier for the years 1861–1955 showed that a very favourable late spring (45 dry days or fewer during October–December) could be expected about once in 8 years. However, it appears that at least 2 such years in succession are needed before *O. fasciculata* can reach plague numbers; this is likely to happen only once in about 77 years. There have been only 11 very favourable late springs during the past 95 years, and only one occasion, 1947–49, when there was a series of such years.

Even though very favourable weather during 1947–49 resulted in a serious outbreak during 1948–50, *O. fasciculata* was scarce in many favourable situations. Unfavourable weather in 1950 (unusually dry late spring) caused a dramatic reduction in the numbers of the species before all of the favourable situations were occupied, and the species returned to its usual condition of being quite rare relative to the amount of food and shelter available in the area.

This study has shown that *O. fasciculata* has become of economic importance because the agricultural dvelopment of County Grey has provided a more favourable environment, enabling the species to increase to greater numbers. One way to reduce these numbers is to eliminate "permanent" pastures and thus provide fewer favourable places where the species may live. By permanent pastures is meant pastures composed of introduced clovers and grasses that are not disturbed by any systematic rotation programme in which paddocks are ploughed and sown to cultivated crops. A rotation programme reduces the area favourable for the species by reducing both the amount of suitable shelter and food.

Another way to make the environment of *O. fasciculata* less favourable is to have as little upstanding dense herbage as possible in the paddocks during the flight season and the egg and early larval stages (September–December). This can be done by concentrating sheep on some paddocks while others are cut for hay and the cuttings removed as soon as possible. Few moths are attracted to fields where herbage is low and so few or no eggs are laid, and the absence of a dense cover of herbage may make these places unfavourable for the species during the late spring and early summer.

If farming practice were changed in this direction it might cause a general reduction in the numbers of *O. fasciculata* in the area. But the relative abundance of the species from year to year would still be determined mainly by the weather during the late winter and late spring.

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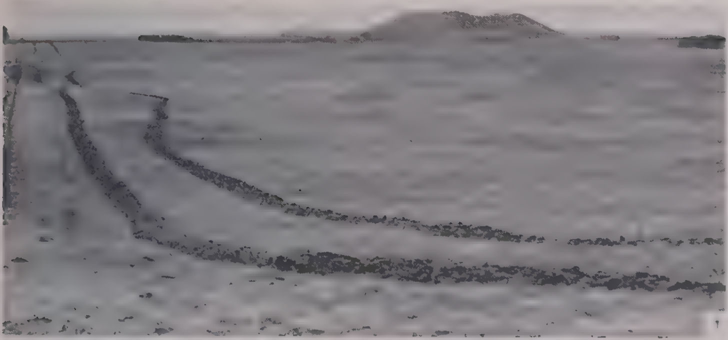
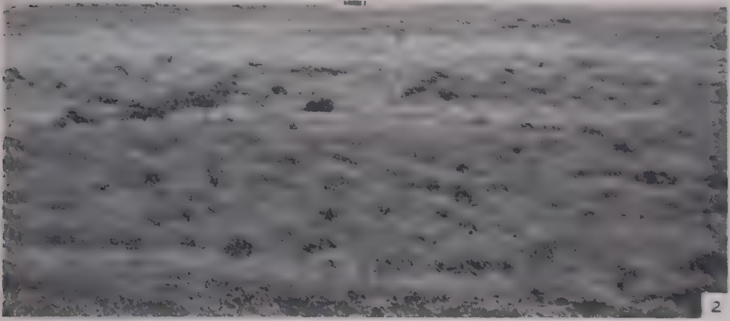
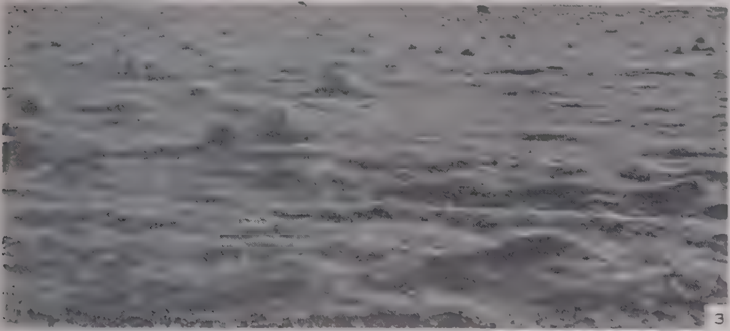
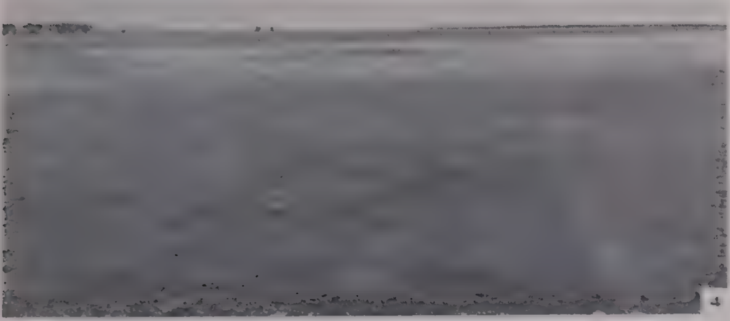
ECOLOGY OF ONCOPERA FASCICULATA. IV









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Explanation of Plate 1

Fig. 1.—An example of pastures severely damaged in 1948. Darker portions of the paddock are where most of the herbage has been consumed; lighter areas are where only old, dead vegetation remains. Mt. Schank is in the middle distance with Mt. Gambier in the background. (Photograph taken by D. C. Swan in September 1948.)

Fig. 2.—Portion of a paddock at Eight Mile Creek severely damaged by *O. fasciculata* in 1949. The lighter patch in the centre has been caused by larvae eating most of the green herbage. (Photograph taken by D. C. Swan in September 1949.)

Fig. 3.—A partially improved pasture containing tussocks of the native white tussock grass, *Poa australis* R. Br., together with introduced grasses and clovers. Under natural, ungrazed conditions, the tussocks are larger and more abundant but the area between them contains little vegetation.

Fig. 4.—Scene showing the influence of surface growth on the abundance of *O. fasciculata*. Herbage in the foreground is low and dense, and no larva was found. In the background the growth is tall and dense, and larvae were in large enough numbers to do severe damage. For further explanation see text.

HYBRIDIZATION BETWEEN *DROSOPHILA SETIFEMUR* AND *D. SPINOFEMORA*

By A. M. Clark*

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Summary

Drosophila setifemur Malloch and *D. spinofemora* Sturtevant can be hybridized in the laboratory without difficulty. From the cross *spinofemora* × *setifemur*, hybrids of both sexes are fertile, but in the reciprocal cross the male hybrids are sterile. In the salivary-gland chromosomes of the hybrids there is no indication of any gross structural differences. Maternal inheritance is suggested as a possible explanation for some physiological differences observed between parent species and the hybrids.

A revised systematic description of *D. setifemur* is presented.

I. Introduction

Interspecific hybridization in *Drosophila* has proved to be a valuable approach to problems of comparative genetics. Although up to 1934 only two cases of hybridization within this genus were known, since then a considerable amount of experimental work has been carried out and the number of cases stands at well over 100 (Patterson and Stone 1952). Besides providing an instance of hybridization between two members of a species group not previously studied in this way, the example to be described here illustrates some points of physiological interest.

D. setifemur Malloch and *D. spinofemora* Sturtevant belong to the *immigrans* species group of the subgenus *Drosophila*, a species group containing about 18 described forms, predominantly from Oriental regions. Since Malloch (1924) first described *D. setifemur*, no further reports of collections of this species seem to have been made. In July 1951, we were able to obtain a few individuals from *Drosophila* collections made with fermenting banana bait in the vicinity of Cairns, Qld. A single impregnated female was isolated and a stock established. *D. spinofemora*, described by Sturtevant (1942), appears to be fairly common in the Hawaiian islands but it does not seem to have spread across the western Pacific to any extent. The stock used in the present work was sent to us by Dr. Marshall R. Wheeler from the collection of *Drosophila* species maintained in the Zoology Department at the University of Texas.

II. General Biology

Both *D. setifemur* and *D. spinofemora* can be maintained in the laboratory on standard culture medium over a temperature range from 18 to 25°C. However, owing to the slow maturation of the female in both species, there is usually a considerable delay before fertilized eggs are laid in new cultures; hence it is essential to include some inhibitor of mould, such as 0·2 per cent. propionic acid, in the culture medium. The use of a mould inhibitor also serves to retard the growth of

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yeast used to seed the cultures, which is advantageous, for prolific yeast growth tends to render the surface of the culture too soft and moist. In neither species do the larvae climb far above the surface of the medium when pupation is about to take place, hence if the culture medium is too soft, many may die.

Both species may be anaesthetized very rapidly with ether. In common with *D. immigrans*, they show a tendency to extrude a drop of urine upon etherization, and this can be troublesome when large numbers of flies are being examined together, owing to one individual adhering to another.

Delay in the development of cultures of *setifemur* and *spinofemora* is primarily the result of the immature state of the females upon eclosion. Both sexes will mate within 24 hr of emergence, but the ovaries are quite immature at this time and fertilized eggs are not laid until the females are about 6 days old, the sperm being stored meanwhile in the spermathecae. Maturation of the ovaries and deposition of eggs can be delayed up to 10 days if the females are deprived of fresh yeast, but owing to the precocious mating, virgin females must be collected every 10 or 12 hr if genetical investigations are being made.

Courtship in *setifemur* is prolonged. In 15 cases in which copulation was observed to take place, the preliminary courting extended over more than ¾ hr. When initiating courtship, the male circles with wings extended and vibrating. Moving in front of the female and with wings making occasional scissor-like movements, he approaches and taps her fore legs. If mating is to be refused, the female lowers her abdomen, flutters the wings rapidly, and moves away. If the courtship is to be accepted, the female herself shows scissor-like movements of the wings. Before finally allowing the male to mount, she extends the wings and holds them motionless at right angles to the body while she rubs her genital region with one of the hind legs. The male meanwhile circles behind her, usually but not invariably licks her genitalia, then mounts and clasps her thorax beneath the wings with his fore legs. The duration of copulation is usually about 35 min the observed range being from 20 to 43 min.

On several occasions in which two or more males were courting the one female, it was observed that after the female had suddenly changed position and moved away, the two males would continue their courtship but directed to each other. There were the customary circling and vibrating movements, but as soon as they had tapped each other on the fore legs, the courtship was immediately broken off until a female was again encountered. The observations suggest that contact with the fore legs leads to recognition of another individual as being of the same or opposite sex.

The mating behaviour of *spinofemora* does not seem to differ in any observable way from that of *setifemur*, nor were there any apparent deviations from the usual pattern of courting behaviour when heterogamic matings were under observation.

III. Reproductive Isolation and Hybridization

The heterogamic pair-mating method was used to detect the occurrence of sexual isolation. Before the tests, both males and females were collected as virgins and aged separately on yeasted culture medium for 6 days. Single pairs of flies were then placed without etherization into culture tubes for 48 hr, after which the spermathecae of the females were examined for the presence of sperm. Of 43 *spinofemora* females, 14 proved to have been inseminated, while of 46 *setifemur* females, 12 were found to have been inseminated. Thus under these conditions, mating between the two species will take place without much difficulty. When reared on standard culture medium at 25°C. successful single pair-matings gave the mean counts of offspring as set out in Table 1. In no case is there a significant deviation from an expected 1 : 1 sex ratio.

Attempts to cross *setifemur* and *spinofemora* with *immigrans* failed. No courtship was observed when *immigrans* males were placed with *spinofemora* and *setifemur* females, while conversely, *setifemur* and *spinofemora* males made no attempts to copulate with *immigrans* females.

TABLE 1

SINGLE PAIR-MATINGS BETWEEN D. SETIFEMUR AND D. SPINOFEMORA

Cross	No. of Pairs	Mean Offspring per Pair	
		Female	Male
setifemur × *setifemur*	10	36	35
spinofemora × *spinofemora*	5	33	30
setifemur × *spinofemora*	10	9	10
spinofemora × *setifemur*	6	17	24

From the cross *spinofemora* × *setifemur*, hybrids of both sexes are fertile and interbreed readily. In the reciprocal cross, however, while the F_1 females are fertile the males are invariably sterile. It may be presumed, therefore, that a *setifemur* X-chromosome requires a *setifemur* Y-chromosome as well to ensure a full complement of fertility genes. On the other hand, a *setifemur* Y-chromosome is sufficient with either a *setifemur* X or a *spinofemora* X. This could be explained by supposing that during the evolution of these two species, fertility genes present on the differential part of the Y-chromosome of *setifemur* have become transposed to the X-chromosome of *spinofemora*. That the autosomes are probably not concerned in fertility is suggested by the fact that if a *setifemur–spinofemora* hybrid female is crossed to a *spinofemora* male, approximately one-half of the male offspring are fertile, the other half being sterile.

In both *setifemur* and *spinofemora*, the chromosome complement consists of a large V-shaped element, two medium sized rods, and a small dot. Miss M. M. Gunson (personal communication) finds that in larval salivary glands of hybrids, there is complete synapsis and no indication whatever of any gross structural differences between the euchromatic regions of the chromosomes of the two species.

IV. PHYSIOLOGICAL DIFFERENCES BETWEEN PARENT SPECIES AND HYBRIDS

Apart from the more yellowish and robust appearance of *spinofemora*, there are no marked morphological differences between the two parent species. There are, however, a few physiological differences that are observable without difficulty. The most conspicuous is the rate of development. At 25°C, the life cycle of *setifemur* is remarkably short, about 8 or 9 days, whereas that of *spinofemora* at the same temperature is of about 15 days duration. The developmental rate of hybrids corresponds closely to that of the female parent species. Thus, the *setifemur–spinofemora* hybrids emerge about 10 days after egg laying, whereas hybrids from the reciprocal cross do not emerge until about the 16th day. There is a sex difference in developmental rate of hybrids from a *spinofemora* × *setifemur* cross, the males developing more rapidly, but in the reverse cross both sexes develop at about the same rate. In the parent species, no sexual differences in developmental rate are apparent.

TABLE 2

SENSITIVITY OF D. SPINOFEMORA AND D. SETIFEMUR SPECIES AND HYBRIDS AT 20°C TO PURE CARBON DIOXIDE PREVIOUSLY SATURATED WITH WATER VAPOUR

Material	No. Recovered after 2 Hr Exposure	No. Failed to Recover
spinofemora	44	9
setifemur	61	39
spinofemora–setifemur hybrids	46	4
setifemur–spinofemora hybrids	36	14

spinofemora *setifemur*	$\chi^2=6\cdot8$**	*spinofemora–setifemur* hybrids *setifemur–spinofemora* hybrids	$\chi^2=5\cdot4$**
spinofemora *spinofemora–setifemur* hybrids	$\chi^2=2\cdot0$	*spinofemora* *setifemur–spinofemora* hybrids	$\chi^2=1\cdot7$

** Significantly different at the 1 per cent. level.

A second physiological difference between *setifemur* and *spinofemora* concerns sensitivity towards carbon dioxide narcosis. This sensitivity is not as marked as that which characterizes the carbon dioxide-sensitive strains of *melanogaster* (L'Heritier 1951) but it is sufficiently pronounced to be readily demonstrable. After a 2-hr exposure to pure carbon dioxide saturated with water vapour, approximately 80 per cent. of the adults of *spinofemora* will show complete recovery, whereas little more than 50 per cent. of *setifemur* individuals will tolerate the treatment The difference is statistically significant. In hybrids, a maternal influence is again apparent, for the *spinofemora* × *setifemur* offspring are the more resistant (see Table 2).

As a final example of a physiological difference between the two species, data on the frequency of wing beat will serve. In conjunction with measurements of wing dimensions, beat frequency has been used on numerous occasions to demonstrate racial or specific differences between various drosophilines (Reed, Williams, and Chadwick 1942; Stalker and Carson 1947). As far as wing dimensions are concerned, there are no statistically significant differences between *setifemur* and *spinofemora*, as reared under laboratory conditions, but there is a difference between the two species with regard to beat frequency. However, in contrast to the preceding physiological differences showing a pronounced maternal inheritance, wing-beat frequencies of the hybrids were found to be intermediate between those of the parent species (Table 3). This is in agreement with the views of Reed *et al.* (1942) who believe that the inheritance of wing-beat frequency is of the multifactorial type.

TABLE 3

WING-BEAT FREQUENCY OF D. SPINOFEMORA AND D. SETIFEMUR SPECIES AND HYBRIDS AS DETERMINED WITH A STROBOFLASH

Material	Mean Frequency of Complete Beats (hundreds/min)	No. of Determinations	*t*	*P*
spinofemora	82·41	14	3·7	0·01
setifemur	91·74	14		
spinofemora–setifemur hybrids	88·22	15	1·8	0·05
setifemur–spinofemora hybrids	83·70	15		

V. DISCUSSION

From the relative ease with which these two species may be crossed, as well as from the close similarity of form, it is clear that divergence has not proceeded very far. The only other member of the group available for hybridization tests was *immigrans* itself, but in view of the considerable morphological differences between this species and the other two, it is not surprising that, under the conditions of the laboratory test, successful heterogamic inseminations between *immigrans* and either *setifemur* or *spinofemora* could not be obtained. More promising perhaps would be attempted hybridization between *setifemur* and *spinofemora*, on the one hand, and *hexastriata* on the other. The chromosomal configuration of the latter species appears to be similar to that of *spinofemora* and *setifemur* (Tan, Hsu, and Sheng 1949).

The most interesting fact revealed by this study is the demonstration of a strong maternal influence in the determination of some of the physiological characteristics of the hybrids. The similarity between the developmental rates of the hybrid and the female parent species has its counterpart in the differences in cleavage rates that Moore (1933) observed in some echinoderm hybrids, where maternal influences were also found to be of importance. No evidence has yet been obtained to suggest that the sensitivity of *setifemur* towards carbon dioxide is related to the presence of a cytoplasmic viroid, hence maternal inheritance is to be regarded provisionally as the most likely explanation for this physiological difference as well. It is probable that if a systematic search were made, other examples of maternally- or cytoplasmically-inherited characteristics of a physiological nature would be discovered readily in *Drosophila*. Rasmuson (1952) has found that the differences in the resistance of various strains of *melanogaster* to narcotics, such as ether, appear to depend, at least in part, on an autonomous cytoplasmic system. However, no evidence for differences in sensitivity towards ether has been obtained in the cases of *setifemur* and *spinofemora* or for their hybrids.

VI. Revised Description of Drosophila setifemur Malloch

The original description of this species, while adequate for the identification of the form, fails to make reference to certain morphological features that have gradually been accepted by *Drosophila* systematists as being reliable and useful. Therefore a revised description has been prepared, based upon material taken from the laboratory stock maintained in this Department.

Male.—Arista plumose, about 12 branches. The antenna pale yellow, the 3rd segment somewhat darker. Front dark yellow, pollinose. Middle orbital nearer to upper orbital and about half as long as the latter. Second oral bristle nearly as long as the 1st. Palps very pale yellow, 1 prominent terminal bristle, 2 prominent lateral bristles. Carina light yellow, narrow, and only slightly broadened below. Cheeks pale yellow, the greatest width about one-eighth the diameter of the eye. Eyes bright red with light pile.

Acrostichal hairs in 8 rows; prescutellar bristles absent. Anterior scutellars convergent; sterno-index 0·8; mesonotum dark, amber coloured, unmarked in young individuals but with a very faint trident pattern appearing in older individuals. Legs yellow, the apical bristles on the 2nd tibia very large. A row of about 12 short, stout, microscopic setae on the apical half of the anteroventral surface of the fore femur. Short, closely placed bristles on the ventrolateral surface, the apical 2 or 3 distinctly longer than the remainder.

Abdomen pale yellow, each segment with a narrow, brown, apical band fading away before reaching the lateral margin. The bands show a narrow but distinct interruption in the mid line.

Wings dusky with conspicuous clouding along the posterior crossvein. Costal index about 3·4; 4th vein index 1·4; the $4c$ index about 0·65 and the $5X$ index about 1·2. Apex of the 1st costal section with 2 well-developed bristles of similar size. Third costal section with heavy bristles on the basal half. Length of body 3·0 mm, wings 2·8 mm.

Female.—Abdominal bands darker. Body 3·2 mm, wings 2·9 mm.

Eggs.—0·56 mm long, 0·19 mm in diameter. Four filaments, the anterior pair thin, about 0·53 mm long, and the posterior pair thick, about 0·74 mm long, and finely branching distally.

Pupa.—Brown, transparent. Anterior spiracles have 18–20 branches.

Chromosomes.—One large V-shaped element, 2 medium sized rods, and a small dot.

Male genitalia.—The genital arch broad above. The lower part with 8 or 9 long bristles, the upper part with 3 or 4 bristles near the posterior margin. Anal plate with 12–15 short bristles at the tip. The claspers have a rounded process above and are provided with 8 or 9 primary teeth and 4 to 6 very stout marginal bristles.

Affinities.—Belongs to the *immigrans* group.

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